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NUTRITIONAL AND HORMONAL EFFECTS ON OVARIAN MORPHOLOGY AND CARCASS TRAITS IN BROILER BREEDER HENS

by

ROBERT A. RENEMA ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Nutritional and Hormonal Effects on Ovarian Morphology and Carcass Traits in Broiler Breeder Hens, submitted by Robert Allen Renema in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Science.

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DEDICATION

This thesis is dedicated to the memory of
Emilynn Grace Jansen Renema,
who came into this world without breath
on May 21, 1995,
and left a profound mark
on our hearts and in our lives.

ABSTRACT

The effects of feeding level, age, and body size were examined in broiler breeder hens to elucidate their effects on ovarian morphology and lipid partitioning. In Experiment 1, 48 broiler breeder hens were either restricted fed (RF) or *ad libitum* fed (AL) from 37 to 59 wk of age, at which time birds continued with a RF or AL feed regimen or had feed increased (FINC) from RF to AL access (16 birds/tmt). Birds were given either a blank (BL) or an estradiol-17β (E2) implant and received a 5 μCi dose of 1-[^{14}C] palmitate to trace lipid allocation over 72 hr. Estradiol-17β treatment reduced feed intake of FINC-E2 birds by 9.2% compared to FINC-BL birds without affecting BW gain. The FINC feeding regimen increased feed intake by 64% over RF values, resulting in relative liver weight increasing from 1.8% of BW in AL and RF birds to 2.7% in FINC birds. Estradiol-17β treatment increased ovary weight by 9% ($P=0.063$) through significantly increasing mean weight of large yellow follicles (LYF). Radiolabeled lipid allocated to the ovary was deposited at the expense of liver and abdominal fatpad deposition. The feed increase had a greater effect on carcass and plasma lipid parameters than did the *estradiol*-17β treatment.

In Experiment 2, the feeding pattern for 48 broiler breeder pullets between photostimulation (PS) and sexual maturity (SM) was either a standard slow feed (SF) or an accelerated fast feed allocation (FF) regimen. 1-[^{14}C] palmitate was used to trace lipid allocation during sexual maturation, and birds reaching SM quickly (EARLY) or slowly (LATE) were compared. Feeding regimen had no affect on traits measured. Incidence of small atretic follicles (<5mm diameter) increased with time and was inversely related to LYF number. Small follicle numbers were up to 80% greater in LATE than in EARLY birds. Body weight was correlated with LYF number in EARLY birds and *estradiol*-17β concentration was correlated in LATE birds.

In Experiment 3, 30 birds were fed a standard (STD) or concentrated (CON) ration between PS and 40 wk of age, when they received either a BL or an E2 implant. Laying sequence length was shorter and pause length was longer in CON than in STD birds between 32 and 40 wk of age. CON birds had more LYF in multiple hierarchies at processing than STD birds.

Low plasma VLDL-TG:PL ratios were associated with higher ovary and stroma weights, and lower fatpad weights than high TG:PL ratio birds. VLDL-TG:PL ratio may be an indicator of reproductive fitness in birds of this age.

In Experiment 4, standard BW (STD), low BW (LOW), and high BW (HIGH) birds were fed either AL or RF between PS and SM. The AL birds reached SM 25.3 d after PS and had 11.0 LYF compared to 38.9 d with 7.1 LYF, respectivley, in RF birds. Small follicle atresia was low in AL birds (10.3), but increased with time to SM in RF birds (mean = 32.3) and was inversely related to LYF number. LH and FSH concentration peaked higher in AL than in RF birds, but remained elevated for a greater period in RF birds, and possibly related to the time-based development of a LYF number control mechanism. Relative fat content and absolute reproductive tract weights were similar across body size groups within feeding regimens.

These experiments demonstrate the extreme sensitivity of the ovary to both nutritional and time effects near sexual maturity, and a weakening of the reproductive response to nutritional status as the birds age. Lipid partitioning to storage sites or metabolic needs is done at the expense of ovarian allocation, and may possibly be predicted by the TG:PL ratio of the plasma VLDL lipids.

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LIST OF ABBREVIATIONS

BW	body weight
AL	*ad libitum*
RF	restricted feed
FF	fast feed
SF	slow feed
STD	standard
CON	concentrated
LOW	low BW
HIGH	high BW
EARLY	first 50% in production
LATE	last 50% in production
PS	photostimulation
SM	sexual maturity
BL	blank implant
E2	estradiol-17β implant
FSH	follicle stimulating hormone
LH	luteinizing hormone
GnRH	gonadotropin releasing hormone
POF	post ovulatory follicles
LYF	large yellow follicles
SYF	small yellow follicles
LWF	large white follicles
MWF	medium white follicles
VLDL	very low density lipoprotein
TG	triglyceride
PL	phospholipid

1. INTRODUCTION

1.1 BACKGROUND

Broiler breeder hen management in the 1990's is becoming a system of ovary management to maximize and maintain adequate egg production. The broiler breeder is a unique type of poultry because it has the genetics for rapid and efficient growth, but it needs to have good egg production to supply the next generation of broiler chicks. However, there is a negative relationship between BW and egg production in broiler breeders (Siegel and Dunnington, 1985), Japanese quail (Marks, 1985), and turkeys (Nestor *et al.*, 1980). Heavily growth-selected male-line turkey hens may be the most reproductively unfit hen in poultry production, with expected rates of lay being about half that of the reproductively-selected female-line hen (Hocking, 1992). Whereas broiler breeders lay better than turkeys, their expected egg production is half that of the very productive egg-type hens.

Modern broiler breeders can grow at three times the rate of a 1957 random bred strain due solely to increased genetic potential (Havenstein *et al.*, 1994). The genetic potential of the broiler chicken offspring of these birds has increased at an estimated rate of 3 percent per year (McCarthy and Siegel, 1983), allowing them the potential to reach market weight in approximately one day less every year (Gyles, 1989). Broiler breeder stocks are now routinely restricted fed from an early age to reduce reproductive problems relating to selection for growth. The need for feed restriction and for refinements to restriction programs are becoming increasingly important as growth potentials in broiler breeder stocks continue to increase at the expense of reproductive potential. An increased level of follicular recruitment and the resulting number of ovarian large yellow follicles (LYF) due to genetic change and overfeeding are the source of the reproductive problems in broiler breeder stocks.

Understanding the ovarian function of the chicken and its interaction with nutritional status, age, and strain is likely the most important issue affecting poultry breeding companies today. The process involves the conversion of genetic, environmental, and nutritional cues into a cascade of signals from the neuroendocrine system. These signals must be integrated and responded to by the organs and tissues primarily involved in reproduction, which will in turn produce more signals for both local and distant activities. The resulting eggs produced are the net result of the bird's attempt to coordinate the demands its body and environment have placed on it. As complex as the control of reproduction is, poultry producers have managed their birds well using what is currently known about lighting and nutrition. However, as poultry meat breeds continue to be further genetically selected for growth parameters, the birds will become increasingly unable to effectively integrate growth and reproductive demands. The industry has progressed to the point where an understanding of the molecular mode of action of the neuroendocrine signals may become more beneficial and even necessary for the future maintenance of reproductive potential in these birds.

This review examines the processes relating to reproductive function in poultry, with particular emphasis on the steroid hormone control of gene expression. Finally, the interaction of reproductive physiology and nutrition as it relates to egg production potential will be examined.

1.2 THE OVULATORY CYCLE AND EGG PRODUCTION

Reproductive Morphology and Follicular Growth

The single functional ovary of the broiler breeder lies at the left, cranial region of the abdominal cavity anterior to the left kidney and caudal to the left lung (Burke, 1984: Gilbert and Wells, 1984). In a normal bird, preovulatory follicles are arranged in a hierarchy of size and represent the majority of ovary weight. A typical hen ovary will contain six to eight large yellow yolky follicles (LYF) (>10 mm in diameter) (Gilbert, 1971), although LYF number can range from four to 12 or more follicles with variations in bird age and feeding level (Yu *et al.*, 1992a). These follicles are typically identified by size and proximity to ovulation (F1, F2, F3, and so on), with the largest follicle being named F1. The smaller follicles have been classified as small yellow follicles (SYF) (5-10 mm in diameter), large white follicles (LWF) (2-4 mm in diameter), and small white follicles (SWF) (<1 mm in diameter) (Robinson and Etches, 1986). Of the 2500 follicles visible to the naked eye on a typical ovary of a domestic hen (Gilbert, 1971), there are several thousand SWF, 10 to 20 LWF, and five to ten SYF. The number of follicles from 1 to 8 mm in diameter is greater in broiler breeders than in the domestic hen (Hocking *et al.*, 1987) and can potentially be altered by feed restriction (Hocking *et al.*, 1989). With increased small follicle numbers, there is an increased incidence of small follicle atresia (individual follicles degenerating through apoptosis and being reabsorbed by the ovary) (Hocking *et al.*, 1987). The majority of follicles undergoing atresia will do so during the slow growth phase of the small follicles (Gilbert *et al.*, 1983). Under normal conditions, the follicle will likely ovulate once it is larger than 8 mm in diameter, or part of the LYF hierarchy.

Soon after the largest follicle has been ovulated, a new follicle is thought to be recruited from the slow-growing small follicle pool into the rapid growth phase of the LYF. However, the follicular hierarchy likely extends down to follicles in the 1 to 3 mm size range, as indicated by their orderly growth patterns, so follicle recruitment is likely occurring at this level (Gilbert and Wells, 1984). Although the process of recruitment has not been well defined, it is known to occur during a narrow time period (Zakaria *et al.*, 1984) and is therefore likely subject to the regulatory mechanisms of the ovulatory cycle. The follicle is transferred into the rapid growth phase weighing between 0.6 g (Bacon *et al.*, 1972) and 0.7 g (Zakaria *et al.*, 1984) and requires 7 to 11 d to pass through the yellow yolky stage in the chicken (Gilbert, 1971; Grau, 1976), or between 11 and 13 d in the turkey (Bacon *et al.*, 1972).

Ovulation is the culmination of a hormonal cascade. It occurs when the F1 follicle ruptures along the stigma, a linear avascular area on the follicle, and the ovum is released from the ovary. The infundibulum, which is the uppermost region of the oviduct, uses its thin, lightly muscularized tissue to engulf the ovum and funnel it into the oviduct (Burke, 1984). The ovum takes 3 to 4 hr to pass peristaltically through the magnum, where egg albumen is released due in part to mechanical pressure from the moving ovum (Moran, 1987). Shell membranes are added to the forming egg during the 1.5 hr it needs to pass through the isthmus. Final 'plumping' occurs when fluid is added to the albumen in the shell gland, and a calcium carbonate and glycoprotein matrix is secreted to form the shell. Chicken eggs require roughly 20 hr in the shell gland, followed by a period of a few seconds to pass through the vagina to complete the oviposition process (Burke, 1984).

Physiological Control of Egg Production

Current understanding of the neuroendocrine and ovarian hormonal control of reproductive development and control of ovulation comes from work with the domestic fowl. It is assumed that similar processes exist in broiler breeder hens. The ovulatory cycle of the chicken is about 25 hours, with a range of 21 to 28 hours (Etches *et al.*, 1984; Etches, 1990). This results in a sequence of eggs being laid, with each egg arriving later in the day than the previous one. Ovulation is restricted to an 8 to 10 hour period of the day, which limits the range in which eggs are sequentially laid to a similar time frame. This 'open period for LH release' is regulated by photoperiod (Wilson and Cunningham, 1984). Consecutive sequences are separated by one or more pause days on which no egg is laid. The ovulatory cycle is controlled by two physiological systems which give rise to its asynchronous nature (Fraps, 1965). The independent systems are the circadian rhythm controlling the open period, and the process of maturation of the F1 follicle. In order for ovulation to occur, the F1 follicle must be steroidogenically competent, and must reach this state within the open period.

A follicle reaches maturity when it acquires the ability to produce sufficient progesterone to stimulate the release of gonadotropins (Robinson *et al.*, 1993a). Progesterone production in a follicle begins when the follicle reaches 8 to 10 mm in diameter (Johnson, 1993). However, this progesterone does not make it into the bloodstream in appreciable concentrations until the follicle reaches the F1 stage, when the follicle loses the ability to convert progesterone into androgens. The progesterone production by the now mature F1 follicle peaks 6 to 8 hours prior to ovulation (Johnson and van Tienhoven, 1980) and triggers gonadotropin-releasing hormone (GnRH) release by the hypothalamus. This in turn is followed by an increase in luteinizing hormone (LH) and follicle stimulating hormone (FSH) release from the anterior pituitary. The LH stimulates an even greater output of progesterone by the granulosa cells of the F1 follicle (Etches, 1990), completing the positive feedback loop producing the LH peak 4 to 6 hours prior to ovulation (Johnson and van Tienhoven, 1980). Results from experiments using steroid synthesis inhibitors indicate progesterone precedes and initiates the LH surge, which leads to ovulation (Johnson and van Tienhoven, 1984). If a follicle acquires the ability to release progesterone outside the open period, the hypothalamus is not responsive and the follicle can wait from 10 to 28 additional hours in an ovulable state. Too great of a delay beyond the acquisition of maturity by the follicle can result in failure to ovulate, which results in follicular atresia (Gilbert *et al.*, 1983). Under conditions of excess LYF numbers, internal ovulation or multiple ovulations can also occur. The failure of the infundibulum to capture an ovulated follicle results in internal ovulation. Multiple ovulations result in multiple-yolked eggs or eggs with shell defects due to insufficient time in the shell gland. These are both considered unsettable egg types due to their extremely limited hatchability.

1.3 INITIATION OF REPRODUCTION

Photostimulation

The natural signal for poultry species to initiate reproduction is the increasing day length of spring. Domesticated species can be photostimulated by increasing the artificial light period to signal the onset of reproduction. In birds, the photoreceptor is in or near the hypothalamus, with the suprachiasmatic nucleus being a likely mediator, as this is the key component of mammalian biological clocks (Moore, 1983). The light signal triggers the production and release of gonadotropin-releasing hormone (GnRH-I) from the anterior hypothalamus. GnRH-I is released into the anterior pituitary, where it stimulates gonadotropin secretion (Sharp, 1993). GnRH-II is also being produced by the hypothalamus, and builds up slowly throughout the egg production period. When daylength begins to decrease, or is no longer able to maintain stimulatory effects on GnRH-I, the inhibitory effects of GnRH-II will be expressed and through a series of events the ovary will gradually regress and egg production will cease (Sharp, 1993).

Role of Sex Steroids

Progesterone acts at the level of the ovary, and at the hypothalamus to stimulate the LH surge for ovulation. Estrogen has limited local action in the gonads but has stimulatory effects on other organs, tissues, and metabolic pathways which directly affect the gonad and the reproductive state. Plasma estrogen concentrations increase within 4 days of photostimulation, peak just prior to sexual maturity, and then drop by about 30% to a level maintained (excluding age effects) for the duration of the laying period (Bacon *et al.*, 1980). Although nuclear estrogen receptors have been localized in chicken granulosa cells (Kamiyoshi *et al.*, 1986), neither estradiol nor progesterone show proliferative effects at this site (Yoshimura and Tamura, 1988).

Following estrogen injection, total bound blood calcium increases due to a tightly binding phosphoprotein associated with yolk precursor transport (Urist *et al.*, 1958). Other early reports demonstrate an increase in total lipids, phospholipids, and free fatty acids after estrogen treatment (Bacon *et al.*, 1980). Plasma very low density lipoprotein (VLDL) concentrations increase about five-fold within 7 days of photostimulation. Estrogen is quickly able to stimulate gene transcription in the oviduct and liver -- the two key areas for production of yolk precursors and egg components. It has a 'self-priming' effect at the level of the granulosa cells, stimulating enhanced steroid production and improving follicular responsiveness to gonadotopins (Shoman and Schachter, 1996). Estrogen acts as part of a positive feedback loop with LH release. LH from the anterior pituitary stimulates estradiol secretion by the ovary, which then induces further

LH release via stimulation of hypothalamic GnRH (Dickson, 1989) by priming the neurons for an enhanced receptiveness to progesterone.

Estrogen concentration is correlated with fatty livers in poultry (Akiba *et al.*, 1982), and receptors have been located in human abdominal fat stores (Prins *et al.*, 1996). If stored fat acts as a hormone sink, a high fat content in poultry may interfere with sex steroid metabolism by taking estrogens out of active circulation. However, it is not known if poultry have estrogen receptors in their major adipose stores as well, which would indicate a functional purpose of estrogen pooling in fat.

LH/FSH Receptor Structure and Function

FSH, LH, and TSH are all synthesized by the anterior pituitary as non-covalently linked dimers consisting of a 92 amino acid α chain and a 118, 115, or 112 amino acid β chain for FSH, LH, and TSH, respectively. The variable β chain likely binds to the hormone receptor on the different target cells and the common α chain mediates activation of the GTP-binding protein (G protein) that associates with the receptor-ligand complex. As in mammals, FSH is the primary factor initiating steroidogenesis in hen granulosa cells at the time of follicle selection (Li and Johnson, 1993a) and is believed to be responsible for follicular recruitment and growth (Stabenfeldt and Edqvist, 1989; Palmer and Bahr, 1992) due to its proliferative effects on granulosa cell numbers and follicular growth. Unlike mammalian LH, avian LH does not luteinize, but rather promotes steroidogenesis and triggers ovulation (Etches, 1990). LH has been shown to stimulate some degree of granulosa cell proliferation in addition to stimulating granulosa cell progesterone producing ability (Yoshimura and Tamura, 1988). At photostimulation, plasma LH concentration increases from 1.5 ng/mL to 6 ng/mL (Wilson and Cunningham, 1980), which then stimulates ovarian growth and steroid production (Robinson and Etches, 1986).

These peptide hormones are synthesized as part of a longer polypeptide that is proteolytically processed to the mature and active molecule before or just after it is transported into the secretory vesicle. A GnRH signal causes immediate exocytosis of the peptide hormone into the blood and stimulates synthesis of replacement hormone stores. (Darnell *et al.*, 1986). The signaling cell typically has one to several days supply of hormone stored in secretory vesicles just under the plasma membrane.

One of the most immediate intracellular biochemical responses to many extracellular signals is protein phosphorylation, in which the enzymatic addition of a phosphate group to one or more amino acid side chains of a protein by a protein kinase. This causes a conformational change and radically alters its activity. Whereas some membrane receptor molecules have intrinsic protein kinase activity, others act by transducing the signal across the membrane with G proteins. The LH and FSH receptors are part of the G protein-coupled family. The receptors feature seven transmembrane predominantly hydrophobic helices, an extracellular N terminus that includes sites for N-linked glycosylation, and an intracellular C terminus that has several serines and threonines for phosphorylation (Kutchai, 1993).

Upon binding a hormone molecule, the activated receptor will associate with a G protein complex consisting of a Gα, Gβ, and a Gγ subunit. A GDP is bound to the Gα subunit of the complex. The Gα subunit is specific to the target receptor molecule and the common Gβ and Gγ subunits act to bind the complex to the plasma membrane. When a hormone-receptor complex interacts with the G protein, it undergoes a conformational change and becomes active. The bound GDP is exchanged for GTP, which frees the Gα-GTP subunit from the complex to interact with adenylate cyclase in the membrane, and strongly stimulates production of the second messenger, cAMP. The Gα subunit hydrolyzes its bound GTP to GDP, and then reassociates with the Gβ-Gγ-dimer (Genuth, 1993; Moran *et al.*, 1994). The G protein mechanism for signal transduction greatly amplifies the original hormonal signal because so many intracellular second messenger molecules are generated. A small number of second-messenger pathways transduce these signals from a wide range of receptors and trigger an equally great variety of cellular responses. Although each receptor is coupled to a particular pathway, there are many points of intersection to allow the cell to integrate the signals it is receiving. The protein kinase C pathway, for example, is known to be involved in the inhibition of steroid production of theca tissue (Johnson, 1993) and is activated by increased cellular calcium concentrations. Recent work with avian granulosa cells has demonstrated that LH stimulates progesterone production through a calcium-permissive cAMP pathway rather than a calcium-dependent pathway. However, the cells also produce other non-steroidal products needed for follicular maturation which are secreted by the calcium-dependent process of exocytosis (Morley *et al.*, 1996).

1.4 STEROID HORMONES

In contrast to the plasma membrane messenger system of the polypeptide hormones, steroid hormones enter the cell and bind their receptors within minutes. Steroids may up-regulate or down-regulate transcription of enzymes, structural proteins, receptor proteins, transcriptional proteins for other genes, or proteins destined for export from the cell (Genuth, 1993). Through the production of these specific regulatory proteins, steroids are able to produce a graded effect by inducing or repressing rather than simply activating or inactivating gene transcription upon binding to its receptor. The steroid system allows for a very precise integration of messages from many effectors.

Steroid Biosynthesis

Steroids are synthesized from cholesterol through a series of discrete enzymatic reactions. The intermediate products of the sex steroid pathway (as with other pathways) may have hormonal properties of their own. Steroid-producing cells typically store a few hours' supply of precursors. Although the hormones may be compartmentalized in the cell, they are not stored in storage granules as polypeptide hormones are. When stimulated, the cell converts the hormone precursors to the finished hormone, which has the ability to diffuse across the plasma membrane into the blood (Genuth, 1993).

The cholesterol precursor for steroid production can be supplied to the steroid biosynthesis pathway in several ways which can vary with both species and cell type involved. The cholesterol supply can come from preformed cholesterol taken up from the blood in circulating lipoproteins, from preformed cholesterol stored within the cell in membranes, lipid-based cholesterol esters, or free cholesterol, or cholesterol can be synthesized *de novo* from products of carbohydrate, lipid, or protein metabolism (Gore-Langton and Armstrong, 1988). Steroidogenic cells are characterized by intracellular lipid deposits containing esterified cholesterol and mitochondria with the cholesterol side-chain cleavage enzyme (P450scc) on the inner mitochondrial membrane (Miller, 1988). This critical enzyme enables the rate limiting, hormonally regulated conversion of cholesterol to pregnenolone (Miller, 1995; Stocco and Clark, 1996). There are associated Golgi bodies for formation of progesterone storage granules and smooth endoplasmic reticulum (SER) with the enzymes P450 17-hydroxylase-lysase for conversion of progesterone to testosterone, and P450 aromatase for conversion of testosterone to estradiol-17β (Shoham and Schachter, 1996). Several dehydrogenase enzymes are present for irreversible conversion from the hydroxylated hormone forms. Estrogens are ultimately formed from either androstenedione or testosterone as an immediate precursor. The P450 aromatase-catalyzed reaction involves three hydroxylations and consumes three NADPH and oxygen molecules (Shoham and Schachter, 1996). The phenolic A ring resulting from this conversion is the structural feature primarily responsible for high-affinity binding to estrogen receptors (Jordan *et al.*, 1985).

A developing follicle is made up of a growing ovum covered with a monolayer of granulosa cells, and then the thecal cell layers, which are divided into the theca interna and theca externa. Following photostimulation, a chicken will begin to produce sex steroids from its thecal cells. In the small follicles on the ovary at this time, the thecal cells are capable of producing dehydroepiandrosterone (DHEA), which can act as a substrate for the elevated concentrations of androstenedione and estradiol-17β observed in the small follicles. The small follicles are, and will continue to be, the hen's primary source of androgens and estrogens

(Robinson and Etches, 1986), as the production of estrogen in the thecal tissue decreases as the follicle matures (Bahr *et al.*, 1983). This process may be due to a reduced number of FSH receptors being present on the follicle as it matures (Ritzhaupt and Bahr, 1987), resulting in a decreased capacity to bind FSH and a reduced conversion of androgens to estrogen (Etches, 1984). Once a follicle reaches 8 to 12 mm in diameter and is recruited into the preovulatory hierarchy, its granulosa cells acquire the ability to respond to LH by producing progesterone (Johnson, 1993), which is then converted to testosterone in the theca interna, and possibly to estrogen in the theca externa (Robinson and Etches, 1986; Saito and Shimada, 1996). The acquisition of the ability to secrete progesterone signifies a change from the Δ5 to the Δ4 steroidogenic pathway within the follicle membrane. Once the follicle reaches the dominant spot in the follicular hierarchy (F1), the thecal cells lose the ability to convert progesterone, resulting in progesterone release and ultimately the positive feedback loop between progesterone and LH causing the LH surge leading to ovulation (Etches *et al.*, 1983).

In most species, FSH and LH are the principle promoting agents for steroidogenesis, production of the enzymatic mRNA, and follicular growth, although the growth factors, insulin-like growth factor 1 (IGF-1), transforming growth factor α (TGFα), and epidermal growth factor (EGF) have also been implicated. In poultry, FSH of mammalian or avian origin has little, if any, effect on steroid production of granulosa or thecal tissue from follicles of any size (Robinson *et al.*, 1988). It does, however, maintain its mitogenic capabilities. In contrast, LH may exert regulatory effects on steroid production through the use of multiple forms (Robinson *et al.*, 1988) differing in their action and specific activity.

Sex steroids can be metabolized into other biologically active forms. Although this occurs primarily in the liver, peripheral tissues can also do the interconversion. For example, in human females, the majority of estrone originates from metabolism of estradiol-17β in various tissues rather than from the ovarian primary source (Stabenfeldt and Edqvist, 1989). This reversible conversion to a less potent form is catalyzed by 17β -hydroxysteroid dehydrogenase (17-HSD). In chickens, the predominant estrogen form during the egg production period is estradiol-17β, whereas in turkeys the predominant form is estrone (Bacon *et al.*, 1980), which estradiol-17β mimics closely at a lower concentration. During sexual maturation, several unidentified forms of estrogen appear to be critical for normal development. The estrone and estradiol-17β forms become essential once daily estrogen cycling begins, however, and remain critical for the duration of the breeder period (Bacon *et al.*, 1980).

Steroidogenic Acute Regulatory (StAR) Protein: The rate limiting step of sex steroid synthesis is the P450scc-mediated conversion of cholesterol to pregnenolone. However, experiments with enhanced substrate and amplified enzyme levels have demonstrated that although this enzyme is notoriously slow, it is the delivery of free cholesterol substrate across the mitochondrial membranes that is actually rate limiting (Miller, 1995). Steroidogenic Acute Regulatory (StAR) protein, a hormone-induced 30 kDa mitochondrial protein plays an essential role (Clark et. al., 1995). This protein is only expressed in steroidogenically active cells of tissues showing an acute response to trophic hormones (gonad, adrenal), and not in steroidogenic tissues (placenta, brain) not exhibiting this response (Miller, 1995). StAR protein begins as a 37 kDa cytosolic precursor protein which is targeted to the mitochondria by its amino terminal signal sequence, imported, and processed to its mature form (Clark and Stocco, 1996). Although StAR protein has not been identified yet in poultry, it is very likely present as well in the gonadal and adrenal organs.

The aqueous space between the outer and inner mitochondrial membranes is the major hindrance to cholesterol translocation. Once cholesterol is delivered to the mitochondrial membrane, it may be passed to the inner mitochondrial membrane possibly either by formation of a lipid bridge or by using StAR as a cholesterol binding protein (Clark and Stocco, 1996). The second messenger pathways shown to increase expression of StAR protein are cAMP-dependent PKA, PKC, and intracellular calcium. StAR is converted to its active phosphorylated state following a trophic exposure to an activating hormone, which then results in an increased steroidogenic response (Clark and Stocco, 1996). A lack of the StAR protein can result in such conditions as lipoid congenital adrenal hyperplasia (human), which is characterized by a complete lack of steroid production (Layman, 1995).

Blood Transport of Steroid Hormones

Sex Hormone Binding Globulins: All hormones initially enter the plasma pool, where they can circulate freely as most polypeptide hormones do, or bound to specific carrier proteins as steroid and thyroid hormones do. Blood albumin is able to form a low affinity bond with hormones. Albumin has a high hormone carrying capacity because of its high concentration (Stabenfeldt and Edqvist, 1989). However, as steroids are hydrophobic, being transported tightly bound by a high affinity carrier protein would be both efficient and protective. These carrier proteins, called Sex Hormone Binding Globulins (SHBG), can significantly extend the plasma life of the hormone by shielding it from structural degradation, clearance through the kidney, or by other processes which efficiently limit the useful life of polypeptide hormones to minutes. Due to the protective nature of the SHBG, steroids have a lifetime of up to several hours in plasma, although metabolic turnover rates of closer to 30 minutes are more common (Genuth, 1993; Stabenfeldt and Edqvist, 1989). Corticosteroid Binding Globulin (CBG) is a similar protein which is responsible for progesterone transport (Hammond, 1993).

SHBG are produced in the liver and exist in biological fluids as a 90 kDa homodimer using a calcium cation to stabilize the structure (Hammond, 1995). Presumably formation of the dimer also forms the active binding site (Hammond, 1993). Androgens were more tightly bound by SHBG than estrogens in many species examined. As only free hormone is considered to be biologically active, SHBG concentrations influence both the distribution and clearance of sex steroids. However, tissues and cells may be able to get around the problem of limited free hormone through specific interactions with the SHBG in tissue areas with differing temperature, blood flow rates, lipid composition of target cell membranes, or location of target cell steroid receptors (Hammond, 1993), thereby allowing an increased efficiency of interaction with SHBG.

As both estrogen and testosterone carrying SHBG appear to interact with surface SHBG-receptors in some tissues, it is becoming clear that the SHBG may be playing a much more active role in their fate (Joseph, 1994). The implications of these bound SHBG being bound to in this form have yet to be elucidated (Rosner, *et al.*, 1992).

The SHBG concentration has implications for both health and lipid metabolism. The sex steroids are considered to be the main physiological regulators of this protein. Their concentration increases with estrogen treatment and decreases with androgen treatment (Toscano, *et al.*, 1992). Additionally, plasma insulin concentrations can be affected by feeding levels and inversely relate to SHBG concentrations in a body weight and fat content-dependent manner (Botwood *et al.*, 1995). This effect, along with the associated increased free testosterone concentrations, can be reversed with reduced calorie intake. Heavy turkey strains have reduced sex steroid concentrations compared to their smaller counterparts (Melnychuk *et al.*, 1994). Perhaps altered insulin concentrations and sex steroid metabolism are contributing to the negative reproductive effects of their lipid allocation patterns.

Hormone Clearance: Metabolic degradation of hormones occurs by enzymatic processes including proteolysis, reduction, oxidation, hydroxylation, decarboxylation, and methylation. The initial degradation of polypeptide hormones can occur after internalization into target cells. In this system, only the receptor may possibly be recycled. Virtually all hormones are extracted from the plasma and degraded to some degree by the liver. Steroids can undergo glucouronidation or sulfation, which alters their structure by rendering them water soluble in both bile and urine (Stabenfeldt and Edqvist, 1989), allowing them to be efficiently cleared from the circulatory system. Hormone metabolites are easily excreted in the urine because they are generally unbound or only loosely bound to protein. The SHBG-bound steroids are unaffected by the kidney as only a small percentage of the bound hormone fraction is filtered by the glomeruli (Genuth, 1993).

The steroid clearance action of the liver is mediated by a multispecific bile salt transporter. These sodium-independent organic anion transporting polypeptides (OATP) have a broad and charge-independent specificity which includes steroid hormones (Hagenbuch and Meier, 1996). This sinusoidal (or basolateral) transporting polypeptide may account for all charge-independent steroid clearance that occurs in the liver. The K_m for the clearance of the anionic steroid conjugates estrone-3-sulfate and estradiol-17-glucuronide were 4.5 and 3.0 μM, respectively (Bossuyt, *et al.*, 1996). The OATP are also present in the kidney (Hagenbuch and Meier, 1996), which is where a high proportion of estrogens are reported to be excreted (Shoham and Schachter, 1996). Hepatic metabolism of estrogen can be enhanced by exposure to chemical pollutants, resulting in lower unbound estrogen levels (Lobo and Cassidenti, 1992).

Steroid Hormone Receptors

Although steroids are able to easily diffuse through the lipid bilayer of the plasma membrane and permeate all cells in the body, cellular response to these hormones are limited to target tissues containing specific cytoplasmic steroid receptors. The concept of signaling by steroid hormone receptors appears simple enough -- these intracellular proteins are activated upon binding hormone and proceed to regulate gene transcription. The minimalist scheme would involve hormone binding to the receptor and causing a conformational change enabling the receptor to now bind DNA and modulate transcription. However, gene transcription is under very tight control and both receptor-ligand binding and receptor-DNA binding are modulated by numerous associated transcription factors. Some genes, such as the gene for the P450scc enzyme, have multiple hormone receptor binding sites in the promoter region to allow transcription in the presence of a variety of stimuli.

Hormone dose-response curves tend to be sigmoidal in shape. The responsiveness of the hormone effect is modulated by receptor or functional cell number, or by the concentration of an essential precursor to the final product or enzyme being activated by the hormone. The sensitivity (hormone concentration needed for half-maximal effect) is influenced by the affinity of the receptors, concentration of modulating cofactors, rate of hormone degradation, and antagonistic hormones (Genuth, 1993). The potency difference between various estrogen forms can be traced to the length of time the estrogen-receptor complex occupies the nucleus. Higher rates of dissociation of the weak estrogens such as estrone and estriol can be compensated for by increasing application to allow extended nuclear binding activity (Shoham and Schachter, 1996).

Steroid hormone receptors are structurally organized in different domains. They have a variable N-terminal region, a small, well-conserved cysteine-rich central region, and a fairly well-conserved C-terminal region (Beato, 1989). The cysteine-rich area is compatible with formation of two zinc-fingers (a single Zn tetrahedrally attached to four cysteines and forming a finger-like protrusion). The well conserved 70 amino acid sequence of the zinc-fingers in the central domain are responsible for the DNA binding activity of the receptors (Beato, 1989; Freedman, 1993). A three amino acid sequence at the base of the first finger determines discrimination of DNA binding, whereas the second C-terminal domain binds the hormone (trans-activation domain) (Lucas and Granner, 1992). The N-terminal end includes the dimerization domain.

Steroid Binding: Role of Heat Shock Proteins: The sex steroid receptors are referred to as ligand-activated transcription factors because the bound receptor is able to interact with the gene directly rather than using a second messenger system. In the absence of hormone, the steroid receptor monomers (glucocorticoid, androgen, estrogen) are maintained in a conformation ready to respond to signal. This form of the receptor is associated with a complex of heat shock proteins (Hsp). Not only do these accessory proteins maintain the monomer in a functional state to bind to their ligand, they also block the receptor's active binding site so it is restricted from interacting with the steroid-responsive promoter of the gene (Beato, *et al.*, 1995). Mutant receptors lacking the domain for binding the Hsp90 or ligand are transcriptionally active, demonstrating the importance of the role of the aporeceptor complex role in keeping unliganded receptors inactive (Bohen *et al.*, 1995). The Hsp are believed to have both protein folding and chaperone functions. They were discovered when steroid receptors from hormone-free cells could be recovered as large 9S complexes whereas receptors from hormone-treated cells would sediment at 4S (Pratt, 1993). The Hsp complex is so stable that it will remain in an equilibrated, associated form even in the absence of receptor (Bohen *et al.*, 1995).

The Hsp-receptor complex consists of a receptor monomer, a dimer of Hsp90, and a Hsp70, and a Hsp56 (Bohen *et al.*, 1995). Poultry also contain a unique p54, p50, and a p23, although it is not clear how they associate with the receptor complex (Pratt, 1993). The Hsp90 interacts with the hormone-binding domain, generating a 'docking' conformation in glucocorticoid and mineralocorticoid hormone receptors. The progesterone, estrogen and androgen receptors also form a stable complex with the Hsp, but do not require Hsp for steroid binding conformation (Pratt, 1993). Whereas the glucocorticoid receptor complexes are predominantly maintained in the cytoplasm in their unbound form, the unliganded sex hormone receptor complexes move immediately to the nucleus from their cytoplasmic site of synthesis and remain in a 'docked' conformation with Hsp90 until hormone binding occurs (Pratt, 1993).

Activation of Transcription: A conformational change occurs in the steroid receptor upon binding steroid which affects the kinetics of the interaction between the receptor and the DNA (Beato, 1989). The Hsp are lost and the receptor-hormone complex dimerizes. A final step before activation is the phosphorylation of the aporeceptor complex (McDonnell, 1995). The progesterone complex is phosphorylated in two stages: one phosphorylation occurs upon displacement of the Hsp, and the second upon association of the receptor with DNA (McDonnell, 1995). Virtually every step in steroid receptor function, from hormone binding to transcriptional modulation and receptor recycling, has been postulated to be influenced by changes in receptor phosphorylation state. Protein Kinase A is essential for activation of the aporeceptor complex by phosphorylation (Denner *et al.*, 1990).

The dimer form of the steroid aporeceptor binds to a specific stretch of DNA in the promoter region called a hormone response element (HRE) (Lucas and Granner, 1992). Although this consensus sequence is similar for some different steroids, it is highly specific for estrogen. The estrogen response element shows very little sign of mutation compared to those of the other hormones. This would suggest that estrogen signaling is essential to life, although life can exist without it (minus reproductive capability). Multiple copies of HRE exist in some genes. The tandem HRE for one of the vitellogenin (yolk protein precursor produced in liver) genes function synergistically to form a more stable complex (Lucas and Granner, 1992).

A final obstacle for proper initiation of transcription can be the chromatin structure of the DNA. A nucleosome of the highly ordered chromatin structure partially blocks access to part of the promoter region. Therefore, in conjunction with hormone induction, there is some chromatin remodeling. Specifically, a soluble complex of gene products can be recruited to partially disassemble the nucleosome, exposing and removing a pair of histone proteins from the histone octet present (Beato *et al.*, 1995; Truss *et al.*, 1996). Access to the masked HRE would now be possible because of the destabilizing effects of the soluble gene product complex on the histone structure. Now the promoter is able to associate with its full complement of transcription factors, associate with RNA polymerase II, and commence gene transcription.

1.5 GROWTH FACTOR RECEPTOR STRUCTURE AND FUNCTION

The superfamily of growth factors, which include IGF-1, EGF, and FGFα, regulate cell proliferation by stimulating resting cells to begin cell division. These receptors have a characteristic tyrosine kinase-mediated signal transduction system. The receptors are proteins with a large, glycosylated extracellular domain, a narrow single hydrophobic transmembrane sequence, and an intracellular protein kinase catalytic domain. EGF-receptor has a cysteine-rich repeat for its extracellular domain, and is responsible for binding both EGF and FGFα. The binding of the receptor triggers dimerization of the EGF aporeceptor, which activates receptor through a conformational change, allowing it to activate protein-tyrosine kinase activity (Kutchai, 1993).

The activated protein-tyrosine kinase receptors can now undergo autophosphorylation by using ATP to phosphorylate the hydroxyl group of specific tyrosine residues within the receptor. Phosphatidylinositol 4,5-bisphosphate (PIP_2), a phosphorylated inner cytosolic membrane phospholipid, can now be formed, with the activation of protein kinase C and increased concentrations of cytosolic calcium ultimately occurring. The phosphoryl groups can be removed from both the receptors and the proteins by protein tyrosine phophatases, hence reducing the activity of the tyrosine kinase system (Moran *et al.*, 1994). Early work in the chicken has demonstrated evidence for EGF and TGFα stimulation of plasminogen activator production in the granulosa cells using a protein kinase C process (Johnson and Tilly, 1990). Further details on the control of this cellular messenger system have not yet been elucidated.

Control of Ovary Development

Poultry granulosa cell proliferation is stimulated by EGF (Yoshimura and Tamura, 1988), TGFα, and IGF-1 (Onagbesan *et al.*, 1996). As in mammals, both EGF and TGFα have been localized predominantly in less differentiated cells in poultry (Van Nassauw *et al.*, 1996). *In vitro* TGFα concentration and receptor numbers decrease with granulosa cell maturation, whereas IGF-1 concentration increases. The TGFα is produced by theca cells and IGF-1 is likely produced in the granulosa (Onagbesan *et al.*, 1996).

Both EGF and TGFα are actively involved in the maintenance of the ovarian small follicle pool in chickens. Small follicle (8-12 mm) granulosa cells contain mRNA for production of the enzymes necessary for conversion of cholesterol to testosterone and estrogen. Whereas granulosa cells *in vitro* will respond to an FSH signal by producing the appropriate mRNA and enzyme product using a cAMP-based pathway, *in vivo* this is blocked by EGF and TGFα. However, upon recruitment into the preovulatory pool of follicles, the inhibitory effects of these growth factors are lost (Li and Johnson, 1993a, 1993b). These growth factors act to prevent premature expression of progesterone conversion enzyme activity and premature differentiation in the granulosa cells of follicles yet to enter the rapid growth phase of follicular development. In contrast to EGF and TGFα action, IGF-1 enhances LH-induced progesterone secretion. However, this effect becomes more prevalent as the follicles approach ovulable size (Onagbesan *et al.*, 1996).

The processes of follicular recruitment is poorly understood beyond the hormonal indicators. Understanding how the effects of EGF and TGFα are modulated and disrupted as follicular recruitment occurs has implications for application in growth selected poultry strains with their excessive follicle complement. Recent work has demonstrated that growing follicles of a broiler breeder strain are more sensitive to endogenous gonadotrophins than in egg-type hens at the critical stage of follicular recruitment to the preovulatory hierarchy (Hocking and McCormack, 1995). This may contribute to the increased rates of follicular recruitment observed in broiler breeder strains and may relate to an altered modulation of follicle response by EGF and TGFα.

1.6 INTERACTION OF NUTRITION AND REPRODUCTION

Hypothalamus and Pituitary

Neuropeptide Y (NPY) has been identified in several species as a stimulator of LHRH release and a potentiator of LH release in response to LHRH (Kalra and Crowley, 1992). Estrogen receptors have been co-localized with NPY-producing neurons in the hypothalamus (Sar *et al.*, 1990) and estrogen potentiates NPY's effects, either directly or by removing the inhibitory opioid peptide influence (Kalra and Crowley, 1992). The preovulatory progesterone surge is believed to drive NPY production directly in the median eminence of the chicken hypothalamus, whereas estrogen and possibly testosterone are thought negatively influence the tone of the opioids (Advis and Countijoch, 1993).

GnRH production potential examined in growth-limited female lambs demonstrated reduced LH secretion (Ebling *et al.*, 1990). It was reported that the low LH concentrations were due to central mechanisms controlling the release, rather than the synthesis of GnRH, and that the nutritionally-based inhibition was not mediated by the opioid system. The median eminence of the chicken hypothalamus responds to feeding level by altering the concentrations of both GnRH-I and GnRH-II. In feed-restricted birds, GnRH-II concentrations increase beginning at 16 to 22 weeks of age, whereas GnRH-I concentrations are increased in birds fed *ad libitum* (D'Hondt *et al.*, 1996). As mentioned previously, GnRH-II concentrations can gradually increase to the point where they interfere with ovulatory control. Interestingly, recent research has revealed that both ovarian granulosa and thecal cells have GnRH-II receptors, and GnRH-II was shown to stimulate steroidogenesis and proliferation in both cell types by way of a receptor-mediated mechanism (Wang and Su, 1996).

Very Low Density Lipoprotein and Lipid Allocation

When previously restricted fed broiler breeders are allowed *ad libitum* access to feed, they will initially overeat, and after 4 d, the daily feed intake will normalize at a higher level than restricted fed birds (Robinson *et al.*, 1993b). Following 7 d of overfeeding, there is growth in all potential lipid depots (ovary, liver, abdominal fatpad). Whereas the *ad libitum* fed birds have a greater excess of energy availability than restricted fed birds to supply lipid synthesis and storage processes, it is not clear if they are partitioning it differently than restricted fed birds do because weight increases in all potential lipid depots. Increased plasma lipid availability may simply have a concentration effect in its increased rate of deposition, or lipid partitioning may shift due to changes in the control of lipid synthesis and/or composition. Plasma estrogen, for example, stimulates hepatic fatty acid and triglyceride (TG) synthesis and the formation of surface lipids to package the characteristically smaller VLDL particles destined for ovarian uptake (Walzem, 1996). Liver-synthesized VLDL for ovarian deposition has a smaller diameter than other VLDL (Griffin *et al.*, 1982) due to a reduced TG content (Walzem, 1996).

Bacon *et al.* (1982) observed that as total plasma lipids in laying quail increased, the proportion of membrane phospholipids dropped slightly. It was theorized that this may be representative of slightly larger lipoprotein particles in the plasma VLDL lipoprotein fraction. The presence of VLDL particles in these birds with a low triglyceride:phospholipid (TG:PL) ratio would then be indicative of a reduced particle size, as more surface material (PL) is needed to package a smaller amount of lipid material (predominantly TG) (Chapman, 1980; Bacon *et al.*, 1982). Griffin *et al.* (1989) injected VLDL from broiler chickens selected for high 7 wk plasma VLDL into low VLDL selected birds and found the clearance rate to be identical to that of native VLDL, demonstrating that differences in metabolism of VLDL in these lines was not due to the VLDL itself. They were using juvenile birds, however, which are not indicative of what occurs in the hen due to the vastly more concentrated plasma VLDL lipid content (Yu *et al.*, 1976) and altered VLDL composition (Fujii *et al.*, 1985) in the mature bird. Studies of metabolic clearance are also not complete without examining tissue uptake, as this may demonstrate differences in the allocation of available VLDL. Research examining the diameter of VLDL particles of birds under differing feeding conditions or laying status demonstrate that a particular range of VLDL diameters appear to be associated with higher rates of reproductive efficiency (Walzem *et al.*, 1994; Walzem, 1996).

Under conditions of extremely positive energy balance, the fate of lipid carried in the plasma VLDL fraction is determined in part by its own composition. Besides its influence on lipid metabolism in the liver through modulation of metabolic hormones, the VLDL concentration can restrict its usage in various tissues. In studies by Fielding (1976) and Fielding *et al.* (1977), the apparent K_m of Lipoprotein Lipase for VLDL in perfused rat heart tissue was found to be much lower than that of perfused adipose tissue, favoring uptake of VLDL by adipose tissue only when VLDL concentrations were high. Griffin *et al.* (1989) reported broiler data that appear consistent with this work, but did not show differences in preliminary analysis of Lipoprotein Lipase K_m values. Genetic selection may also affect affinity for plasma VLDL. Analysis of chicks of a layer strain shows a reduced rate of plasma VLDL clearance and tissue oxidation in birds selected for high 7 wk plasma VLDL concentration (Griffin *et al.*, 1991). Comparison of lean and fat Leghorn strains demonstrates that the plasma fatty acid release in the presence of lipoprotein lipase is greater in the fatter strain due to differences in susceptibility of plasma VLDL to hydrolysis by lipoprotein lipase (Griffin and Hermier, 1988). In broiler lines selected for high or low plasma VLDL concentration, Griffin *et al.* (1989) found that the fatter, high-VLDL birds deposited radiolabeled VLDL into the abdominal fatpad at a rate 2.5-fold greater than that of the low-VLDL birds. Although broiler plasma lipid data may not be applicable to breeders, VLDL and plasma lipid concentration can clearly affect where they are deposited or utilized.

A potential consequence of overfeeding broiler breeders is the excessive accumulation of lipid in the liver. The rate of lipid synthesis rather than the rate of mobilization is altered in a feed-restricted state (Leclercq, 1975). Overfeeding chickens is believed to saturate the very low density lipoprotein (VLDL) plasma lipid carrier synthesis and transport system, resulting in liver accumulation of triglyceride (TG) (Leclercq *et al.*, 1974). If the production of TG by the liver surpass its ability to form VLDL, excess TG can be temporarily stored in cytoplasmic triglyceride-rich vesicles (TGRV) (Mooney and Lane, 1981). The half-life of plasma VLDL from chronically overfed birds has been reported to be increased as indicated by a slow turnover rate of the enlarged lipid pool of the liver (Bacon *et al.*, 1978). As more than 90% of fatty acid synthesis has been reported to occur in the liver of poultry (Leveille *et al.*, 1975), the overfeeding of birds can have substantial effects at the level of the liver, with metabolic conditions such as fatty liver hemorrhagic syndrome (FLHS) being expressed if liver lipid mobilization cannot keep pace with synthesis.

Factors Affecting Egg Production

Broiler breeder hen candidates are routinely restricted fed from an early age to circumvent reproductive problems brought about by selection for growth. Excessive BW and lipid content in broiler breeder hens negatively affects settable egg production, fertility, hatchability, and egg shell quality (Yu *et al.*, 1992; Robinson *et al.*, 1993a). The ovaries of growth-selected strains are sensitive to overfeeding during reproductive development. Extra feed at this time will result in excess production of LYF likely to be arranged in multiple hierarchies (Hocking *et al.*, 1987, 1989; Katanbaf *et al.*, 1989; Yu *et al.*, 1992), thereby increasing production of unsettable eggs (Jaap and Muir, 1968). Rates of multiple ovulation are controlled through feed restriction, which is associated with a subsequent increased rate of settable egg production (Hocking *et al.*, 1987; Yu *et al.*, 1992).

The laying pattern of eggs can have implications for potential egg production within a bird. The prime sequence length (characteristically long sequence early in production) has been observed to increase as rate of lay increases in both broiler breeders (Robinson *et al.*, 1991a) and turkeys (Lerner *et al.*, 1993). Whereas in turkeys this is also related to an increased sequence length (Anthony *et al.*, 1991; Lerner *et al.*, 1993), in broiler breeders, the extra egg production is related to a reduced inter-sequence pause length (Robinson *et al.*, 1990). Overfeeding birds results in the production of shorter sequences than in feed restricted birds (Robinson *et al.*, 1991a). In birds undergoing longer sequences, the pause length between consecutive sequences is reduced (Etches, 1990). Following a pause, the first of sequence egg has been found to be heavier than subsequent eggs (Robinson *et al.*, 1991b), presumably because of increased yolk size due to follicular sequestering of yolk right up to the time of ovulation (Etches *et al.*, 1983). This has implications for chick size, as higher yolk weights and yolk:albumin ratios normally associated with increased age (O'Sullivan *et al.*, 1991) also result in increased hatch weight (Shanaway, 1984).

Broiler breeders fed *ad libitum* from photostimulation have an average of 12 LYF at sexual maturity compared to 7 LYF in restricted-fed hens (Hocking *et al.*, 1987; Yu *et al.*, 1992).

The adverse effects of *ad libitum* feeding occur primarily before peak production (Yu *et al.*, 1992). By 44 wk of age, overfeeding hens will increase LYF production within 14 d, but to a lesser degree than at sexual maturity (Robinson *et al.*, 1993b). By 54 wk of age, McGovern *et al.* (1997) found that increasing feed allowance did not alter ovarian morphology. Hocking (1996) speculated that the reduced response of the ovary to increasing feed intake as birds age was due to a reduced sensitivity of the ovary via a reduced interaction of nutritional status with the reproductive hormones. In younger birds, a sudden increase in feeding level is reported to result in a disproportionately high accumulation of carcass fat, increased LYF weights, and an increased incidence of double LYF hierarchies after a 14 d experimental period (Robinson *et al.*, 1993b). Although the LYF number did not differ after 7 d of overfeeding, overfed birds had a higher incidence of multiple hierarchies, indicating that in certain cases the number of LYF on the ovary may be less important than the uniformity of spacing of the follicles in ensuring the more regular, single ovulation of follicles (Robinson *et al.*, 1993b). Increasing feed access to *ad libitum* levels was believed to relate to a loss of regulation of the ovarian hierarchy within a few days of overfeeding (Robinson and Wilson, 1996).

Robinson *et al.* (1995) found that a one follicle increase in birds on an accelerated feeding program during the period of sexual maturation was associated with a 10 egg reduction in total egg numbers over the breeder period. The state of the ovary at sexual maturity may set the tone for reproductive performance for the duration of the breeder period. In birds of similar BW, the group receiving a greater feed allocation will have the higher number of LYF (Hocking, 1993), and will accelerate the sexual maturation process (Wilson and Harms, 1986; Yu *et al.*, 1992). Early maturing birds can have increased LYF numbers (Hocking *et al.*, 1988) and multiple hierarchies (Hocking, 1992; Renema *et al.*, 1997), although the primary effector of LYF numbers appears to be BW (Hocking and Whitehead, 1990; Hocking, 1993, 1996). Heavier broiler breeder strains will have a greater number of LYF than lighter strains (Udale *et al.*, 1972; Reddy and Siegel, 1976).

Nutritional Status and Reproduction

Poultry can be brought into production without a photostimulatory cue with minimal delay (Leeson *et al.*, 1988), suggesting that light is not the only factor involved in reproductive status. Birds will also respond to photostimulatory signals at early ages, but corresponding egg production can be poor, and some poultry types will have difficulty maintaining egg production (Hocking, 1992; Renema *et al.*, 1995). Whereas photostimulation at a young age causes increased plasma LH concentrations within four days, the bird's ovary will typically take much longer to mature than in older birds (Leeson *et al.*, 1988).

Chickens have been demonstrated to respond to photostimulation with reproductive development as early as three weeks of age (standard age is 18 to 20 wk) when maintained on an extremely high fat diet (Dunn *et al.*, 1986). As heavier birds typically come into production earlier and with a greater lipid content (Summers and Leeson, 1983), age at sexual maturity has become considered to relate to body weight and composition within the context of hormonal balance (Leeson *et al.*, 1988). In humans, a minimum content of fat also appears to be necessary for the onset of menarche (Frisch, 1982). In a study of dieting women with unexplained infertility, the LH:FSH ratio in women weighing between 85 and 89% of ideal body weight (IBW) has been reported to be 2.4 compared to 4.6 for women between 90 and 94% of IBW, indicating an interaction between nutrition and FSH production in particular. Successful pregnancy occurred in many women who reached 95% of IBW (Bates *et al.*, 1982). Extreme obesity will increase the LH:FSH ratio further, as well as significantly reduce SHBG concentration (Zhang *et al.*, 1984).

These observations about sexual maturity and lipid stores can be traced to interactions between hormonal impulses for reproductive development and impulses for growth. In poultry, many of these impulses are expressed at the liver level. Chronic positive energy balance will result in high levels of insulin and thyroid hormone (Simon, 1989). The addition of stress can increase corticosterone, which will further intensify triglyceride synthesis and VLDL secretion.

This can occur to the point where feed intake and lipid synthesis increase while the ovary regresses (Etches *et al.*, 1984). Estrogen stimulates the production of specific apoproteins for the maintenance of yolk deposition (Schneider *et al.*, 1990), but the negative effects of hormones like thyroid hormone on the gene expression of this apoprotein can have disastrous effects on the reproductive system (Akiba *et al.*, 1982).

A delay in sexual maturity is known to be associated with feed restriction programs (Pym and Dillon, 1974; Wilson and Harms, 1986; Yu *et al.*, 1992b), which has prompted the simplistic explanation for poultry of a minimum BW requirement for SM (Brody *et al.*, 1980, 1984). There is some variability in this measure, as it has been refined to include body fat (Bornstein *et al.*, 1984) and lean body mass (Soller *et al.*, 1984), and age (Brody *et al.*, 1980, 1984). The work of Soller *et al.* (1984) demonstrated, using a combination of dietary restriction and realimentation dietary nutrient densities, that fat content alone was not sufficient to initiate sexual maturity, but that there may be a lean body mass requirement for sexual maturity based on the uniformity of lean body mass across all treatment groups. This is supported by the work of Zelenka *et al.* (1982) with *ad libitum* fed and restricted fed Japanese quail. Hocking (1996) described a failure of previous research to demonstrate a relationship between body fat and number of LYF, whereas BW is clearly related. This research indicates that fat levels observed at sexual maturity may be inadvertent results of other more important events rather than having a deterministic role in the sexual maturity process, which concurs with the lack of importance of specific fat content in mammalian models (Bronson and Manning, 1991; Wade and Schneider, 1992).

Brody *et al.* (1980) reported a minimum BW requirement of 2300 to 2700 g to be required for onset of lay. As *ad libitum* fed birds reach this weight at 14 to 15 wk of age and yet did not commence lay until 21 to 25 wk of age, a minimum age for sexual maturity was proposed. Attainment of sexual maturity likely depends on age more heavily in *ad libitum* fed broiler breeders and on BW and carcass traits in restricted fed birds (Katanbaf *et al.*, 1989). Differences in the age or physical requirements for sexual maturity can exist between broiler breeder strains due to genetic selection programs. In a study of birds with high and low BW thresholds for reproduction, Eitan and Soller (1993) found the low-line birds entered lay sooner and at a lower BW than high-line birds. They hypothesized the differences were due to decreased photoperiodic drive in the high-line relative to the low-line birds, which may limit the birds' ability to respond to a light signal.

Despite their simplistic nature, the concepts of BW and age thresholds requirements for sexual maturity do appear to work in describing or predicting age or body conformation of chickens at sexual maturity. Clearly the initiation of the complex physiological process of sexual maturation must go beyond an internal BW scale as a trigger, but rather BW and age are acting as indicators of an internal balance between energy balance or nutritional indicators and responsiveness of the reproductive hypothalamo-pituitary-gonadal hormonal axis. In mammals, whole body energy balance is considered a more accurate regulator of ovulation than fat stores (Bronson and Manning, 1991). Normal changes in the body's metabolism during the transition form juvenile to mature adult are thought to influence the rate of maturation of the neuroendocrine system and therefore the timing of puberty (Steiner, 1987). Blood-borne metabolic factors, in particular glucose, amino acids, and insulin, were found to augment LH secretion in immature animals. In their review of metabolic fuels and reproduction, Wade and Johnson (1992) state that reproductive physiology is sensitive to the availability of oxidizable metabolic fuels, and that with reproductive development, ovarian hormones play a major role in the changes in ingestion, partitioning, and utilization of metabolic fuels. The extent of such relationships in avian species have yet to be explored to this extent.

1.7 EFFECTS OF AGING ON REPRODUCTION

The ovulation rate of poultry is dependent on the availability of small follicles for recruitment into the follicular hierarchy. As the birds age, their rate of egg production gradually declines due to increased rates of small follicle atresia (Waddington *et al.*, 1985; Palmer and Bahr, 1992; Johnson, 1993) and a reduced small follicle pool size, resulting in fewer large follicles in the preovulatory hierarchy (Johnson, 1993). In broiler breeders, the problem is compounded in that they have a poorer persistency of lay compared with egg-type hens (Robinson *et al.*, 1993a). Mean sequence length has been reported to decline from a peak of 19.0 d at 32 wk of age to 2.6 d at 62 wk of age (Robinson *et al.*, 1990). The immediate implications of a reduced small follicle pool for hormone production are reduced estrogen concentrations. This in turn may decrease rates of lipid and yolk precursor synthesis through estrogen-stimulated pathways, and possibly contribute to the extended time needed for large follicle maturation through reduced sensitivity of hormone action or production in estrogen target tissues. It is well known that the large follicles are much heavier in old birds than in young birds, and that this difference is partly due to follicles of the older hens developing the competency to ovulate at a slower rate than in young hens (Johnson *et al.*, 1986; Bahr and Palmer, 1989). This may be due to older birds having a reduced capacity to respond to hormone signals both at the follicular (Johnson *et al.*, 1986) and at the hypothalamic level (Williams and Sharp, 1978). As the age-related decline in basal LH was not due to the responsiveness of the anterior pituitary, Williams and Sharp (1978) proposed that the lower LH concentration was due to a reduced action of the neural mechanisms controlling basal GnRH secretion. Treatment of older birds with FSH stimulates follicle production and yolk deposition while reducing rates of follicular atresia (Palmer and Bahr, 1992), indicating a decreased amount of hormone signaling in older birds due to reduced FSH output and/or reduced FSH receptor activity.

1.8 CONCLUSIONS

By this example it is clear that nutritional and hormonal messages are constantly being modulated to suit the current metabolic needs of the bird. The sex steroids, although potent activators of gene transcription, are simply one of many hormonal influences that have implications for reproduction. It is clear that maintaining a reproductive state is a profoundly complicated process. It is incredible to observe how well modern growth-selected poultry strains can lay despite the constant boosting of their growth potential. The nutritional and hormonal environment of the ovaries in these birds is changing and will continue to do so. An understanding of the ovary's response to the influences of these environments will therefore become increasingly important.

1.9 DESCRIPTION OF EXPERIMENTS

Objectives

This research program was designed to examine the effects of feeding level, age, and body size on the ovarian morphology and lipid allocation of broiler breeder females. The specific objectives were:

1. To identify specific traits or physiological events relating to the number of LYF at sexual maturity and late in the egg production cycle.
2. To test the effectiveness of feeding program, BW, or exogenous estrogen treatment for altering LYF numbers.

Experiments

1. **Purpose**: To determine if overfeeding and/or estrogen treatment can stimulate yolk or follicle production in birds near the end of the egg production cycle.

Description: The effects of long and short-term *ad libitum* feeding in combination with exogenous estradiol-17β treatment on egg production, ovarian morphology, and carcass traits were examined in 59 wk old hens. Lipid allocation to the liver, abdominal fatpad, and ovary was traced with radiolabeled palmitic acid.

2. **Purpose**: To examine the specific effects of feeding regimen known to alter LYF number at sexual maturity and of timing of the onset of lay on ovarian morphology, carcass traits, and plasma VLDL characteristics.

Description: Birds were given a standard or an accelerated feed restriction program between photostimulation and sexual maturity and sorted into 'early' or 'late' groups relating to day of first oviposition within each feeding regimen. Ovarian morphology, carcass traits, plasma lipid parameters, and lipid allocation to the liver, abdominal fatpad, and ovary were examined at sexual maturity (first oviposition).

3. **Purpose**: To determine if an experimental high breast-yield strain is reproductively tolerant of overfeeding and if its ovary is responsive to exogenous estrogen at 40 wk of age.

Description: Birds were given a standard or concentrated diet beginning at photostimulation. Production parameters were monitored until 40 wk of age, when blank or estradiol-17β implants were given for a 2 wk period. Effects of feeding regimen and estrogen treatment on ovarian morphology, carcass traits, and plasma VLDL characteristics were examined.

4. **Purpose**: To determine if plasma estradiol-17β, LH, and FSH concentrations are related to ovarian morphology at sexual maturity in low, standard, and high weight birds under standard or *ad libitum* feeding conditions.

Description: Birds of standard BW, or 20% light or heavy were restricted fed or *ad libitum* fed between photostimulation and sexual maturity. Plasma samples taken at 3 d intervals were analyzed for plasma estradiol-17β, LH and FSH concentration. Feeding regimen and body size effects on ovarian morphology, carcass traits, and plasma sex hormone concentrations were examined.

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2. EFFECTS OF ESTRADIOL-17β IMPLANTS AND FEEDING LEVEL ON OVARIAN FOLLICULAR DEVELOPMENT AND LIPID ALLOCATION IN BROILER BREEDERS AT 59 WEEKS OF AGE

2.1 INTRODUCTION

Lipids deposited in ovarian and adipose tissues of chickens are supplied by the very low density lipoprotein (VLDL) fraction of plasma. VLDL concentrations can increase from 60 mg/100 mL plasma in immature birds to 1400 mg/100 mL in sexually mature hens (Yu *et al.*, 1976). Estrogen stimulates liver production of a unique VLDL form destined almost exclusively for ovary deposition. This smaller form of VLDL has a diameter of approximately 30 nm with little variability (Griffin *et al.*, 1982), is resistant to peripheral hydrolysis (Bacon *et al.*, 1978a; Schneider *et al.*, 1990), and is thought to have its tight surface radius stabilized by apoprotein VLDL-II, a yolk-specific apoprotein induced by estrogen (Miller and Lane, 1984).

Research at the University of Alberta has shown that *ad libitum* access to feed early in the egg production period causes substantial increases in both ovarian yolk mass and carcass lipid content (Robinson *et al.*, 1991; Yu *et al.*, 1992a, 1992b). Robinson *et al.* (1993) examined the short term effects of a sudden increase in feed allowance in 44 wk old broiler breeder hens and reported substantial increases in liver weight and fat content, and in plasma lipid concentration after 2 wk *ad libitum* access to feed. Large ovarian follicle numbers and mass also increased, as has been reported in young hens (Yu *et al.*, 1992b). A second experiment carried out at 54 wk of age (McGovern *et al.*, 1997), showed that carcass lipid content increased with either a 10 or 20 g increase in restricted feed allocation and that ovarian morphology was not affected. The potential value of *ad libitum* access to feed in birds near the end of the egg production cycle on ovarian morphology was not assessed.

Near the end of the production cycle egg production declines presumably due to a reduced rate of recruitment of ovarian follicles resulting in follicular insufficiency. Hence, it would be of benefit to find a management procedure to boost or 'flush' ovarian production of yolky follicles for increased egg production in the latter half of the broiler breeder production year. One of the actions of estradiol-17β is the stimulation of production of yolk lipids and proteins by the liver. As plasma estradiol-17β concentration decreases with bird age, supplementing concentrations to a level approximating that of a bird at the onset of lay may be of benefit for the maintenance of follicle and egg production.

This study characterizes the effects of long-term *ad libitum* feeding on rate of egg production, plasma lipid parameters and plasma estradiol-17β concentration, and examines the effects of a physiological dose of estradiol-17β treatment and long or short-term *ad libitum* access to feed on lipid allocation and ovarian morphology late in the egg production cycle. The effects of initial laying rate and lipid parameters on the degree of change in ovarian and plasma lipid parameters following a feed or sex hormone challenge are examined.

2.2 MATERIALS AND METHODS

Stocks and Management

A total of 72 individually caged Shaver Starbro[1] broiler-breeder hens of similar BW were randomly assigned to one of the following feeding regimens at 37 wk of age (36 hens/tmt): AL (*ad libitum*-fed), and RF (restricted-fed). During rearing and to 36 wk of age, birds were restricted fed by limited daily feed allocation following industry guidelines. Beginning at 20 wk of age, birds were fed a breeder diet as recommended by the breeder (Table 2-1). Daily individual egg production data were recorded beginning at 41 wk of age. Laying records were subjected to sequence analysis where a sequence was defined as a period of consecutive ovipositions separated by a pause of one or more days. Mean sequence length was determined by assigning each day a sequence length number based on the total length of the sequence encompassing that day. Mean pause length was determined in a similar fashion. At 59 wk of age all birds were weighed and assessed for laying status. Hens that were determined to be in egg production and within one standard deviation of the feeding regimen BW mean were randomly assigned to an experimental group as follows (16 hens/tmt): AL (continued *ad libitum* feeding regimen); RF (continued restricted feeding regimen); and FINC (feed increase birds switched from RF to AL feeding regimen) (Table 2-2). The 16 birds in each treatment were also assigned to either an estradiol-17β (E2) or a blank (BL) implant treatment (8 birds/tmt). Birds were moved into new laying cages arranged in a randomized block design and managed as before. The experimental protocol was approved by the Animal Policy and Welfare Committee of the Faculty of Agriculture, Forestry and Home Economics of the University of Alberta.

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[1] Shaver Poultry Breeding Farms Ltd., Box 400, Cambridge, ON, N1R 5V9

Experimental Procedures

Hormone Treatment: Solid silastic pellets containing estradiol-17β or no steroid (blank) were manufactured as described by Trudeau *et al.* (1991). The hormone implant size was based on a previous unpublished study of dose and BW effects in AL and RF broiler breeders (Robinson, personal communication). This preliminary work revealed that these implants increased plasma estradiol-17β concentration by 120 pg/mL.

Birds were weighed and an initial 10 cc blood sample was collected for plasma lipid and hormone analysis prior to estradiol-17β or blank Silastic pellet implantation at 0800 h. Birds received either the 2 X 2 X 18 mm hormone pellet (contained 7.2 mg estradiol-17β/pellet) (E2 birds) or a similarly sized blank pellet (BL birds). The central portion of the sparsely feathered area caudal to the cervical feather tract was swabbed with ethanol and anesthetized with a 0.5 cc subcutaneous injection of Lidocaine. The hormone or blank pellet was inserted subcutaneously with curved forceps through a 2 mm incision. A single 2-0 silk stitch was placed to close the incision and eliminate risk of implant loss. Birds in the FINC feeding treatment were then allowed *ad libitum* access to feed, whereas the remaining birds continued on their previous feeding regimen. Individual feed intake was recorded daily.

Lipid Partitioning: Birds were maintained for 24 h following implantation to allow acclimation to the implants and stabilization of plasma estradiol-17β concentrations. A gelatin capsule[2] containing 5μCi of 1-[^{14}C] palmitate[3] diluted with 300 μL of canola oil was then administered orally. The capsule was administered by placing it at the caudal part of the tongue and allowing the bird to swallow it. Birds were maintained on assigned feeding regimens for 72 h, at which time a final 10 cc blood sample was taken for analysis of plasma traits as above. At 0800 h birds were euthanatized by cervical dislocation, and individual BW recorded. The liver, abdominal fatpad, oviduct, and ovary were removed and weighed. The liver and abdominal fatpad were then stored at -15°C until radioactivity was quantified. The number and weight of large yellow ovarian follicles (LYF) (> 10 mm diameter), and the number of small yellow follicles (SYF) (5-10 mm diameter) and large white follicles (LWF) (2-5 mm diameter) were recorded. Follicle size classifications were based on previous reports (Robinson and Etches, 1986), although in the current study the range of diameters used was continuous. Stroma weight was recorded with the LYF removed (initial stroma) and again when the SYF and LWF had also been removed (bare stroma) to assess treatment effects on large and small follicle types separately. An assessment of the potential for multiple ovulations to occur was determined by assigning LYF of similar size (differing by less than 1 g or 1 mm diameter) to the same position in the hierarchy as reported previously (Renema *et al.*, 1995). The incidence of internal ovulation (as evidenced by yolk residue in the body cavity) was assessed. Follicles with a discolored and/or shrunken appearance were considered atretic (Gilbert *et al.*, 1983). The incidence of follicular atresia of the yellow follicles (> 5 mm diameter) was assessed. Following assessment of ovarian morphology, all parts of the ovary were recombined and frozen. Egg yolks from eggs laid during the 72 h following ingestion of the radioactive lipid as well as developing eggs found in the oviduct at processing were frozen separately. The frozen samples were later individually homogenized in a small meat grinder and freeze-dried for 7 d.

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[2] Size No. 1 Empty gelatin capsules, T.U.B. Enterprises, North Augusta, ON
[3] SA = 54 mCi/mmole, NEN Research Products, Boston, MA.

Liver, abdominal fatpad, ovary, and yolk lipids were assessed using standard chemical analysis procedures (AOAC, 1980) on duplicate 1 g (abdominal fatpad) or 2 g (liver, ovary, and yolk) dried samples. True tissue lipid content was calculated by adjusting recorded values to account for moisture loss during the tissue drying process. Isolated tissue lipid was dissolved with 3 mL of petroleum ether following final weighing and sub-sampled into 20 mL scintillation vials (800µL). Petroleum ether was evaporated and sub-sample weight determined. Ten mL of a toluene-based organic scintillation solution (Toluene with 4.0 g/L PPO and 0.1 g/L POPOP) was added to all vials and liquid scintillation counting (LSC) performed with a Packard 1600 CA Tri-Carb Liquid Scintillation Analyzer using a 5 min counting time. Due to a yellow coloring of the ovary and yolk lipid extracts, and a pale brown coloring to the liver extracts, 20 µL of ^{14}C-Toluene[4] was added to each sample as an internal standard to allow for accurate quench correction. An efficiency of 89%, 96%, and 93% was calculated for counts from liver, abdominal fatpad and ovarian tissue, respectively. Sample activities were adjusted to reflect an efficiency of 100%. Ovary and yolk activities were pooled to represent total activity in the ovary during the 72 h radioactive lipid period. Radioactivity incorporated into liver, abdominal fatpad, and ovary tissue was expressed as a percentage of the total absorbed radioactivity.

Blood Plasma Analysis: Blood samples were taken from the brachial vein using EDTA-coated vacuum blood collection tubes and 21 gauge needles. Blood was centrifuged at 1500 *g* for 20 min at 3°C. A preservative solution of streptomycin sulfate and penicillin-G (50 U each/mL stock) was added to plasma at a level of 20 µL/mL to limit bacterial and mold growth. Plasma for subsequent lipid quantification and RIA analysis was stored at -30°C. Plasma undergoing VLDL lipid quantification and analysis was further treated with 1 µL/mL of a 1M benzamidine solution and 5 μL/mL of a 0.2M phenylmethylsulfonyflouride (PMSF) solution to limit protease activity. Two mL of plasma were placed in a Beckman polycarbonate ultracentrifuge tube[5] and overlayered with 2 mL of 0.85% KBr solution (density = 1.0063). Samples were ultracentrifuged in a Beckman 50 Ti rotor at 40,000 rpm for 7 hr at 20°C in a Beckman L8-70M ultracentrifuge. The semisolid layer from the meniscus area of the tube (VLDL layer) and the top half of the tube contents were removed with a pasteur pipette. Fatty deposits remaining on the tube wall were scraped off with a plastic scraper. Samples were stored at -30°C until analysis of VLDL parameters was performed. To reduce lipid adhesion to plastic and glass surfaces, all pipette tips and glassware in contact with the samples were pretreated with Sigmacote[6].

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[4] SA = 8.12×10^5 DPM/g, Amersham

Plasma lipid weights and VLDL lipid weights were determined by Folch lipid extraction. Chloroform was evaporated from the 15 mL extracted aliquot under a nitrogen stream on a hot-plate (70°C at surface) until a constant weight was achieved. Thin layer chromatography (TLC) was used to isolate triglyceride (TG) and phospholipid (PL) lipid classes from the VLDL lipids. Two-hundred μL of chloroform was added to the dried VLDL sample and a 50 μL sample (for LSC analysis) plated at the origin of a lane on a 20 X 20 cm TLC plate. The plates were placed in an 80:20:1 solvent cocktail of petroleum ether, ethyl ether, and acetic acid for 30 min. Plates were lightly sprayed with a 0.1% 8-Anilino-1-naphthalene-sulfonic acid (ANSA) solution and the lipid bands visualized under UV light. The TG and PL bands were marked and scraped off into scintillation vials and LSC performed as described above. However, lipid class samples were counted for 10 min due to low radioactivity levels.

The proportions of VLDL lipid classes were determined with latroscan thin-layer chromatography/flame-ionization detection (TLC/FID) instrumentation[7]. Two sets of ten matched Chromarods were spotted with 0.5 μL (4 to 6 μg) of sample dissolved in chloroform. The solvent system used consisted of benzene (88.9%), chloroform (10.5%), acetic acid (3.9%), and methanol (2.6%). Rods were developed in the solvent cocktail for 1 hr. Upon drying of the rods, the rods were scanned in the Iatroscan TLC/FID Analyzer. The response factors (RF) of the individual rods were calculated by using a mixture of lipid class standards as follows: cholesterol ester (CE) (5%), TG (68%), cholesterol (CH) (3.0%), and PL (24.0%). This cocktail approximated the proportions of the lipid classes in plasma VLDL. The RF calculated for these lipid classes were used to calculate the actual proportions of the lipid classes of all samples measured on each rod.

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[5] Beckman Instruments, Palo Alto, CA 94304
[6] Sigma Chemical Co. St. Louis, MO
[7] latron Laboratories, Inc., Tokyo, Japan

Plasma estradiol-17β concentration was determined by RIA[8] using duplicate 200 μL samples in a single assay. Assay parallelism was determined by measuring estradiol-17β concentration in various plasma volumes. In 50, 100, 200, and 400 μL of plasma, estradiol-17β concentration was (mean ± SEM) 31.0 ± 3.0, 101.8 ± 6.8, 247.6 ± 8.4, and 599.5 ± 14.0 pg/mL. Sample duplicate variation of up to 5% was allowed. The intra-assay coefficient of variation was 5.24%. The assay sensitivity was 1.5 pg/mL. The antiserum was highly specific for estradiol-17β with a relatively low cross reactivity to other naturally occurring steroids in the plasma sample as stated by the manufacturer. All tested compounds had a cross-reactivity of less than 1% with the exception of d-Equilenin (4.4%), Estrone (10%), Estrone-β-D-glucuronide (1.8%), and Ethinyl estradiol (1.8%).

Statistical Analysis

Data collected from AL and RF birds prior to hormone implantation were analyzed using one-way analysis of variance (SAS Institute, 1994). Sequence and pause length analyses were performed by comparison of individual means generated for the time period of 41 to 59 wk of age. Source of variation for the parameters compared at the time of hormone implantation was feeding regimen The experimental design following hormone implantation was a 3X2 factorial with the main effects, Feed and Hormone. Birds were randomly assigned to cages arranged in randomized blocks of six birds. Data collected at processing were evaluated by two-way analyses of variance using the General Linear Models (GLM) procedures of SAS® (SAS Institute, 1994). Sources of variation at processing were feeding regimen, hormone, and the interaction between feeding regimen X hormone. Differences between means were evaluated with Fisher's protected LSD procedure (Peterson, 1985). The error variation for all variables consisted of the variation between birds within the interaction. No effects of block were observed for parameters measured.

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[8] Kit Number TKE25, Diagnostic Products Corp., Los Angeles, CA 90045-5597

Pearson correlation coefficients (Steel and Torrie, 1980) were computed between reproductive, carcass, and plasma parameters across all treatments and within each feeding regimen. Following experimental analysis, birds of all treatments were pooled and ranked into the upper and lower 50% for the parameter, total egg production, to better define the effects of rate of lay on susceptibility to change from a feed or sex hormone challenge. Birds of the upper 50% of the ranking were compared to the lower 50% for each parameter by subjecting them to one-way Analysis of Variance using SAS (SAS Institute, 1994). Four birds were removed from the experiment due to leg problems or ovarian regression. The SEM values presented were therefore based on the treatment group with the fewest birds within each main effect or the interaction. Unless otherwise stated, all statements of significance were based on testing at the $P < 0.05$ level.

2.3 RESULTS AND DISCUSSION

Initial Production and Lipid Parameters

Results of BW, egg production, and plasma lipid parameter comparison between AL and RF birds between photostimulation and hormone implantation (59 wk of age) are presented in Table 2-3. Body weights of the AL birds were greater than those of the RF treatment (4106 *vs.* 3605 g, respectively). Twenty-two weeks of *ad libitum* feeding had resulted in a 14% increase in BW, but not, however, in reduced reproductive efficiency normally associated with excess BW. Settable egg production did not differ between feeding regimens (Table 2-3). The similar egg production values may be due to the age of the birds during the AL period, as the majority of egg production reductions observed in overfed birds generally occur prior to peak production. Absolute yolk weights in AL bird eggs were significantly higher than for RF birds (21.5 *vs.* 19.9 g; respectively), but not different when expressed as percentage of egg weight (31 *vs.* 30% for AL and RF yolk weights, respectively). Egg weight was not significantly greater in the AL than RF birds ($P=0.10$).

Laying sequence analysis for the 41 to 59 wk of age period demonstrated that the AL birds were laying similarly to the RF birds, but with a longer sequence length and a lower number of sequences relative to the RF birds (Table 2-3). By laying more eggs consecutively in fewer sequences, the AL birds were laying fewer first-of-sequence eggs, thereby improving their potential fertility. The total number of pauses were also reduced in AL birds.

Plasma estradiol-17β concentration ($P=0.741$) and plasma lipid parameters ($P=0.171$ to 0.604) did not differ between feeding regimens (Table 2-3). The lipid profile of the RF birds was related to total egg production, however, with the TG:PL ratio of the VLDL lipid fraction inversely correlated with total eggs ($r=-0.49$; $P<0.01$) in individual birds. It has been suggested that a reduced TG content of VLDL may be related to a reduced VLDL particle size (Chapman, 1980; Bacon *et al.*, 1982), although the same authors state that altered TG:PL ratios are minor compared to changes in VLDL particle concentration in plasma lipid shifts. However, the role of the TG:PL ratio in indicating or determining the rate of lay is also supported by Renema *et al.* (1997), in which birds with low VLDL-TG:PL ratios were found to have a heavier ovary and a decreased relative abdominal fatpad weight compared to high VLDL-TG:PL birds. A low VLDL-TG:PL ratio may allow a higher proportion of circulating lipids to be taken up by the yolk rather than be metabolized or stored as adipose tissue which may be due in part to VLDL particle size. Although plasma lipid concentration did not correlate highly with egg production, it was strongly correlated with the VLDL TG:PL ratio ($r=0.62$; $P=0.0008$) and therefore may be related to plasma lipid utilization.

Carcass Traits and Ovarian Morphology

The AL birds were heavier than birds of the RF and FINC feeding treatments at processing (Table 2-4). The change in BW between hormone implantation and processing was significantly greater in the FINC birds (5.8% for FINC *vs.* 0.1% on average for AL and RF birds). Daily feed intake was significantly affected by both feeding regimen and hormone treatments (Table 2-4). The FINC regimen birds consumed 60% more feed per day than their RF counterparts. Estradiol-17β treatment birds consumed 9.2% less feed than BL birds and yet gained the same BW during the experimental period (Table 2-4). Feed intake in the FINC birds was negatively correlated with initial plasma estradiol-17β concentration ($r = -0.770$; $P=0.026$). Birds with a lower initial plasma estrogen concentration were more likely to consume more feed under *ad libitum* conditions than birds with higher estrogen concentrations. This suggests that the negative relationship between plasma estradiol-17β and feed intake may be a natural physiological response in broiler breeders of this age.

Comparison of feed consumption per unit of BW in AL and RF birds showed that they consumed similar amounts of feed (33.7 *vs.* 35.4g feed/kg BW for AL and RF birds, respectively). These feed intake rates may explain the similarities in egg production traits in these treatments and how *ad libitum* access to feed was not detrimental to egg production in AL birds at this age.

Analysis of carcass lipid parameters demonstrated feeding regimen to be a more potent effector than estrogen treatment. The AL birds had larger abdominal fatpads than birds of the RF and FINC treatments (Table 2-4), although this difference probably existed prior to the hormone implant study period. No change was observed in FINC fatpad weight during the 4 d hormone implant period. Liver weight and lipid content were both increased by the FINC feeding regimen. Liver weight of FINC birds was 56% greater than that of their RF counterparts (Table 2-4). FINC bird liver lipid content almost doubled in 4 d, increasing to 58.2% from an average of 34.5 % for the other feeding regimens. On an absolute basis, the 22.4 g of extra feed per kg that the FINC birds consumed each day increased liver lipids from the 22.2 g measured in RF birds to the significantly greater 58.7 g found in FINC birds at processing (P<0.0001). Liver lipid content in AL birds was similar to levels found in RF birds.

Oviduct weight was reduced by estradiol-17β treatment (Table 2-5). E2 bird oviducts were 8 g (11%) lower in weight than those of the BL birds. The difference in oviduct weight between hormone treatments as a percentage of BW approached significance (P=0.06). Whereas there was no relationship between initial estradiol-17β concentrations and oviduct weight, hormone concentrations at processing and the change in concentration were negatively correlated with relative oviduct weight (r = -0.425; P=0.005). Relative oviduct weight was also negatively correlated with BW (r = -0.576; P<0.0001) and positively correlated with relative ovary weight (r = 0.336; P=0.03). Thus the reproductive tract maintained a relatively constant size regardless of bird BW.

Ovary weights of E2 birds were numerically greater by 9.8% (P=0.063) over that of the BL birds (Table 2-5). Hormone treatment appears to have had an opposite effect on the ovary than on the oviduct by slightly increasing rather than reducing its weight. This suggests that yolk deposition was affected by increased circulating estradiol-17β. Mean LYF weight was higher in E2 birds (10.8 g) than in BL birds (9.8 g) (Table 2-6). The weight of LYF was inversely related with BW in RF birds (r = -0.785; P=0.0009), whereas it was positively related in FINC birds (r = 0.566; P=0.04). In restricted fed birds, a smaller body size was an indication of a bird investing its available nutrients into egg production rather than storage. In the previously restricted FINC birds, a larger body size reflected the increased nutrient intake and enabled greater storage of newly available nutrients as demonstrated by the LYF weight with BW correlation. The incidence of atresia was measured as more than twice as high in E2 birds compared to BL birds (P=0.06) (Table 2-6). Follicular atresia of ovarian follicles greater than 5 mm in diameter (LYF and SYF) may be more likely under elevated estradiol-17β conditions, which may have been due to the negative feedback of estradiol-17β on hypothalamic stimulation of follicle stimulating hormone (FSH) and luteinizing hormone (LH) release.

Ovary weight was greater in birds on the AL feeding regimen than on the RF regimen (Table 2-5). Both the initial stroma weight (ovary without LYF) and the bare stroma (ovary without LYF, SYF, or LWF) weighed the most in FINC regimen birds and the least in RF birds. It appears that following 4 d of *ad libitum* access to feed, the stroma of the FINC treatment birds had adopted similar characteristics to those of AL birds (Table 2-5). Treatment differences were more pronounced for bare stroma, indicating that the ovaries of the FINC birds may have been changing primarily at the level of the smaller follicles. Follicle numbers were not significantly different for feeding regimen (Table 2-6), although LWF numbers were numerically greater (P=0.08) in the AL and FINC treatments than in the RF treatment.

Apart from a single case in an AL-E2 bird, there was no incidence of multiple follicle arrangement of LYF into sets of similar size and maturity. Bird age appears to have an effect on the ovary's responsiveness to excess feeding. *Ad libitum* feeding of birds at sexual maturity results in the largest increases in LYF number and incidence of multiple follicle arrangements (Hocking *et al.*, 1987; Yu *et al.*, 1992b). A sudden increase in feeding of birds at 44 wk of age also resulted in the production of multiple hierarchies, but with fewer extra LYF (Robinson *et al.*, 1993). The number of LYF was reported to be less important than the uniformity of spacing of such follicles in ensuring the regular, single ovulation of follicles. Whereas in those overfeeding studies there is a loss of regulation of the ovarian hierarchy in response to excess feeding, the response becomes more gradual in time, and by 54 wk of age (McGovern *et al.*, 1997) there is no longer an affect on LYF number. Hocking (1996) speculated that the sensitivity of the ovary to extra feed availability may be decreasing with broiler breeder age. By 59 wk of age, ovarian LYF numbers do not change with overfeeding, suggesting that the link between nutrition and reproduction is no longer an inducable pathway.

Lipid Partitioning

The distribution of radiolabel between the liver, abdominal fatpad, and ovary was driven primarily by feeding regimen (Table 2-7). Newly available lipid in the over-eating FINC treatment birds appears to be being allocated to the liver at the expense of the ovary. The proportion of radiolabel deposited in the liver was 17.8%, 13.6%, and 36.3% for AL, RF, and FINC birds, respectively, whereas ovary deposition was 48.2%, 51.8%, and 26.2%, respectively. Deposition in the abdominal fatpad did not differ between feeding regimens and averaged 35.4% across all treatments. The proportion of ovarian radioactivity was negatively correlated with liver activity ($r = -0.713$; $P<0.0001$) and with abdominal fatpad deposition ($r = -0.620$; $P<0.0001$), whereas the proportion of liver and abdominal fatpad radioactivity did not correlate with each other. In a study of overfeeding birds at 40 wk of age, Robinson *et al.* (1993) found that the liver was a more predominant site of lipid accretion than the abdominal fatpad following 7 d of *ad libitum* feeding.

Hormone treatment did not have significant effects on lipid allocation when compared across all feeding regimens due to the significant effect of feeding regimen in FINC treatment birds (Table 2-7). However, when birds continuing on the same feeding regimen (AL and RF) are examined without FINC birds, the estradiol-17β implants significantly affected lipid allocation. The proportion of radiolabel in the liver was increased from 11.4% to 19.8% by estrogen treatment ($P=0.03$). This difference may reduce abdominal fatpad deposition (38.9% *vs.* 29.7% in BL and E2 birds, respectively) ($P=0.09$).

In a previous study of lipid allocation, Bacon *et al.* (1978a) injected radiolabeled VLDL into laying hens and after 6 h recovered 20%, 30%, and 25% of injected activity in the liver, ovary, and carcass, respectively, with the remainder of the activity presumably having been oxidized to CO_2. In the current experiment a higher proportion of activity was recovered from the ovary, and a lower amount from the liver. This is likely due to the experimental time-frame, as hens would have a greater opportunity to clear lipid and activity from the liver in 3 d. In a study by Burghell-Mayeur *et al.* (1990), peak values for an ingested radiolabel in a laid egg-yolk was 4 d after administration of the gel capsule. This time corresponds to the 72 h between administering the label and processing the birds in the current experiment, and may explain why a greater incorporation of activity was localized in the ovary. Recovery of total administered radioactivity in the liver, abdominal fatpad, and ovary was 46.5, 51.2, and 46.3% for birds of the

AL, RF, and FINC regimens, respectively. Efficiency of radiolabel uptake did not differ by feed or hormone treatment.

The LYF mass of the FINC treatment birds was not different from those of the other feeding regimens, so a similar amount of lipid was likely being deposited in their ovaries. If ovarian lipid deposition is thought of as a constant, it can be seen that considerably more lipid may have been deposited in the liver and abdominal fatpad than the reported proportions suggest. The plasma lipid carrying capacity of the FINC birds were similar to that of the AL birds, however, as indicated by plasma lipid and VLDL concentrations (Table 2-8). The half-life of plasma VLDL from chronically *ad libitum* fed birds has been reported to be longer than that of short-term *ad libitum* fed birds (Bacon *et al.*, 1978b) which would allow the FINC birds to clear their plasma VLDL more quickly. If the production of TG by the liver is surpassing its ability to form VLDL, the excess TG can be temporarily stored in cytoplasmic triglyceride-rich vesicles (TGRV) (Mooney and Lane, 1981). The livers of FINC birds may have had an unnaturally high proportion of radioactive lipid present because VLDL production was not able to keep pace with TG production, thereby resulting in excessive amounts of TG being stored in TGRV. As the liver enzymes involved in lipid synthesis were not examined in the present experiment, it is not known if the rate of lipid synthesis was similar in AL and FINC birds or across all treatments. In their analysis of lipogenic accessory enzyme activities in overfed laying hens, Walzem *et al.* (1993) found that there was considerable individual variation in the extent of nutritional induction of the enzymes. Leclercq (1975), reported that the rate of lipid synthesis rather than mobilization is altered in a feed-restricted state. Bacon *et al.* (1978b) examined force-fed chickens at 10 wk of age and reported that peak efficiency in converting lipid substrate into carcass lipid occurred 3 d after the onset of force-feeding, which is similar to the time-line of the current study. As more than 90% of fatty acid synthesis occurs in the liver in poultry (Leveille *et al.*, 1975), overfeeding birds has substantial effects at the liver level.

Plasma Estradiol-17β and Plasma Lipid Parameters

Estradiol-17β treatment was used in the current experiment as a method of stimulating yolk deposition and possibly increasing ovarian follicle recruitment. The estradiol-17β implants increased plasma estradiol-17β concentration by 103 pg/mL relative to the BL implanted bird concentration (Table 2-9), which maintained estradiol-17β concentrations at physiological levels and close to the target concentration increase of 120 pg/mL. Hormone means for each group in the feeding regimen X hormone interaction ranged between 145 and 288 pg/mL (Table 2-8). However, hormone treatment had no significant effect on plasma lipid parameters at processing (Table 2-8) or on differences in parameters in the 86 h hormone implant period (Figure 2-9). In their examination of endogenous estradiol-17β production by small white follicles (SWF) (<1mm diameter), Yu *et al.* (1992c) found that increased feed intake enhanced SWF steroidogenic activity. In the current study, although there were no differences in initial plasma estradiol-17β concentrations (P=0.741) (Table 2-3), FINC bird hormone concentrations were numerically increased by 18.5% over AL and RF concentrations at processing (P=0.12) (Table 2-8).

Feeding regimen had no significant affect on plasma lipid traits at processing (Table 2-8), or on the differences in plasma lipid traits during the 86 hr experimental period (Table 2-9). As with carcass morphology traits examined, the FINC treatment was responsible for the greatest numerical differences between feeding regimens. The change in FINC treatment plasma lipid concentration, for example, approached significance with a rise of 13.9 mg/mL compared with 8.6 and 3.9 mg/mL increases in the AL and RF treatments, respectively (P=0.06) (Table 2-9). As with ovarian SYF and LWF numbers, processing plasma lipid parameters (Table 2-8) suggest that FINC birds may be changing to be more like that of AL birds.

A more specific measure of VLDL composition came from analysis of radiolabel distribution between the lipid classes (Table 2-10). Examining the ^{14}C distribution within the VLDL reflects current usage patterns by the bird and does not appear to be confounded by pre-existing conditions such as carcass lipid stores, plasma lipid concentration, or rate of plasma lipid catabolism. The proportion of VLDL-TG and VLDL-PL in the FINC birds changed to proportions similar to those of AL birds. Both AL and FINC regimen TG and PL proportions were significantly higher and lower, respectively, than RF proportions (Table 2-10). The FINC bird VLDL-TG:PL ratio, which was similar to that of the AL birds, was significantly greater than the RF bird ratio. Bacon *et al.* (1978b) found similar trends in the radiolabel incorporation of birds receiving extra feed for 3 d and 3 wk. The feeding regimen X hormone interaction was significant in the current study. Of particular interest was the reduction in TG:PL ratio with hormone treatment within the FINC feeding regimen (Table 2-10). Estradiol-17β implants may be modulating the effects of a change to *ad libitum* feed access in these birds. The estrogen-treated FINC birds also had a numerically reduced BW gain and daily feed intake (Table 2-4). Estrogen is known to stimulate liver production of VLDL of a smaller diameter (Griffin *et al.*, 1982) which is preferentially taken up by the ovarian follicles (Schneider *et al.*, 1990). As mean LYF weight was significantly increased in E2 birds, it would be expected that the VLDL-TG:PL ratio would be lower in these birds as a result of the production of an increased proportion of smaller VLDL particles containing less TG (Chapman, 1980; Bacon *et al.*, 1982). The observed trend of reduced VLDL-TG content may partly explain why average LYF weight was increased by hormone treatment.

As egg production was similar accross treatment boundaries, all birds were sorted by rate of egg production. The top 50% of egg producers had a higher plasma lipid concentration at the start and at the end of the hormone implant period than the bottom 50% of egg producers. The initial plasma lipid concentrations were 23.2 and 17.9 mg/mL for the top 50% and bottom 50% of egg producers, respectively. The number of eggs laid correlated most strongly with the initial plasma lipid concentration ($r = 0.320$; $P=0.03$). The best layers appeared to undergo the greatest change in plasma lipid concentrations as a result of feeding regimen or hormone challenge ($r = 0.310$; $P=0.05$) and, in restricted fed birds, had the lowest VLDL-TG:PL ratio ($r = -0.573$; $P=0.04$). Birds with a low initial TG:PL ratio were also most likely to undergo large increases in TG:PL ratio ($r = -0.656$; $P<0.0001$) primarily through increased VLDL-TG ($r = -$

0.846; P<0.0001). A low TG:PL ratio would be expected to be associated with a reduced VLDL particle size (Griffin *et al.*, 1982) and possibly with increased lipid allocation to the ovary.

The initial plasma lipid parameters appear to be related to final ovarian stroma size in FINC birds. Plasma lipid concentration at hormone implantation was positively correlated with final stroma weight (r = 0.576; P=0.02). The initial VLDL-TG:PL ratio also correlated with stroma weight (r = 0.768; P=0.002) and SYF number (r = 0.692; P=0.009). The FINC birds were the only ones to undergo substantial changes in their feed intake during the experimental period (Table 2-4). It is possible that tissues such as ovarian stroma, with its population of estrogen producing small follicles (Robinson and Etches, 1986), may exert some influence on plasma lipid composition through interaction with lipid synthesis systems in the liver. The role of estrogen in the formation of a yolk-specific population of VLDL particles has been well characterized (Walzem, 1996). Estrogen stimulates hepatic fatty acid and TG synthesis and formation of surface lipids to package the characteristically smaller VLDL particles destined for ovarian uptake (Walzem, 1996). Liver-synthesized VLDL for ovarian deposition has a smaller diameter than other VLDL (Griffin *et al.*, 1982) due to a reduced TG content (Walzem, 1996).

Under conditions of extremely positive energy balance, the fate of lipid carried in the plasma VLDL fraction is determined in part by its own composition. An increased plasma lipid and VLDL concentration can influence how it is utilized by the bird. Besides its influence on lipid metabolism in the liver through modulation of metabolic hormones, the VLDL concentration can restrict its usage in various tissues. In studies by Fielding (1976) and Fielding *et al.* (1977), the apparent K_m of lipoprotein lipase for VLDL in perfused rat heart tissue was found to be much lower than that of perfused adipose tissue, favoring uptake of VLDL by adipose tissue only when VLDL concentrations are high. Griffin *et al.* (1989) report broiler data that appear consistent with this work, but did not show differences in preliminary analysis of lipoprotein lipase K_m values. Genetic selection may also affect affinity for plasma VLDL. A reduced rate of plasma VLDL clearance and tissue oxidation has been shown in birds selected for high 7 wk plasma VLDL concentration (Griffin *et al.*, 1991).

Bird age may be affecting lipid allocation in these birds. As plasma estrogen concentration declines with age and egg production, less of the estrogen-induced precursors for VLDL with low lipoprotein lipase susceptibility will be produced, allowing more lipid to be oxidized by peripheral tissues or stored in lipid depots. Comparison of lean and fat Leghorn strains demonstrates that the plasma fatty acid release in the presence of lipoprotein lipase is greater in the fatter strain due to increased susceptibility of plasma VLDL to hydrolysis by lipoprotein lipase (Griffin and Hermier, 1988). Altered VLDL susceptibility to lipoprotein lipase may contribute to observed differences in fatness. Estrogen is believed to reduce lipoprotein lipase in laying conditions through the inhibition of lipoprotein lipase synthesis (Griffin and Hermier, 1988). In broiler lines selected for high or low plasma VLDL concentration, Griffin *et al.* (1989) found that the fatter, high-VLDL birds deposited radiolabeled VLDL into the abdominal fatpad at a rate 2.5-fold greater than that of the low-VLDL birds. Although broiler plasma lipid data may not be applicable to breeders, VLDL and plasma lipid concentration can clearly affect where they are deposited or utilized.

It has previously been shown that the ovary does not respond to extra feed availability late in production (McGovern *et al.*, 1997). In the current study, increased follicle development did not occur in response to estradiol-17β supplementation. However, hormone treatment did increase lipid allocation to the ovary, as indicated by increased mean LYF weight (Table 2-8), which possibly occurred through the stimulation of production of yolk-precursor VLDL in the liver. Changes to feeding level late in the egg production cycle predominantly affect rates of lipid allocation to storage depots. The results of the current experiment support the conclusions of McGovern *et al.* (1997), who stated that the consequences of overfeeding old broiler breeder hens are not as severe as has previously been reported in young hens. As late as 40 wk of age, overfeeding has been reported to increase LYF number (Robinson *et al.*, 1993). It appears that in older birds the link between nutrition and reproduction which is responsive to overfeeding at a young age is no longer responsive.

TABLE 2-1. Composition and analysis of breeder diet fed from 21 to 60 wk of age

Ingredient and analysis	Percentage (%)
Ground wheat	33.76
Ground corn	14.31
Ground oats	10.00
Soybean meal (48%C.P.)	13.42
Ground barley	15.00
Wheat shorts	1.29
Limestone	7.68
Dicalcium phosphate	1.06
Choline chloride premix[1]	0.50
Layer microingredient premix[2]	0.50
Iodized salt	0.28
L-lysine HCL	0.03
DL methionine	0.17
Tallow	2.00
Calculated Analysis	
ME (Kcal/kg)	2750
CP (%)	15.49
Calcium (%)	3.19
Available P (%)	0.42
Lysine (%)	0.75
Methionine (%)	0.41
TSAA (%)	0.65

[1] Provided choline chloride in the diet at a level of 100 mg/kg.

[2] Provided per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU; cholcalciferol, 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 40 IU; vitamin K, 2.0 mg; pantothenic acid, 14 mg; riboflavin, 6.5 mg; folacin, 1.0 mg; niacin, 40 mg; thiamine, 3.3 mg; pyridoxine, 6.0 mg; vitamin B12, 0.02 mg; biotin, 0.2 mg; iodine, 0.5 mg; Mn, 75 mg; Cu, 15 mg; Zn, 80 mg, Se, 0.1 mg; Fe, 100 mg.

Table 2-2. Breeder period feeding regimen

	Experimental Feeding Treatment[1]		
Age (wk)	AL	RF	FINC
	Feeding Level		
20 to 36	Restricted	Restricted	Restricted
37 to 58	*Ad libitum*	Restricted	Restricted
59	*Ad libitum*	Restricted	*Ad libitum*

[1] AL = *ad libitum* fed; RF = restricted fed; FINC = previously RF birds increased to AL feeding level.

Table 2-3. Production and plasma lipid parameters of *ad libitum* fed (from 37 wk of age) and restricted fed broiler breeder hens at the commencement of the experimental hormone implantation and feeding level period (59 wk of age)

Parameter	Feeding Level[1] AL	RF
	(Mean ± SEM)	
n[2]	16	32
BW (g)	4106 ± 75^{a}	3605 ± 52^{b}
Egg production traits[3]		
Production (%)	68.8 ± 2.3	66.3 ± 1.7
Total eggs	86.6 ± 3.0	83.5 ± 2.1
Settable eggs	85.9 ± 3.1	82.3 ± 2.2
Egg weight (g)	69.8 ± 1.3	67.1 ± 0.9
Yolk weight (g)	21.5 ± 0.4^{a}	19.9 ± 0.3^{b}
Sequence analysis		
Number	30.1 ± 1.4^{b}	36.9 ± 1.0^{a}
Average length (d)	2.94 ± 0.18^{a}	2.22 ± 0.13^{b}
Pause analysis		
Number	30.0 ± 1.4^{a}	36.6 ± 1.0^{b}
Average length (d)	1.45 ± 0.11	1.25 ± 0.08
Plasma traits		
Estadiol-17β concentraion (pg/mL)	151 ± 14	151 ± 10
Plasma lipid concentration (mg/mL)	22.2 ± 2.2	19.8 ± 1.6
Plasma VLDL		
VLDL lipid concentration (mg/mL)	17.3 ± 1.9	14.1 ± 1.3
TG:PL ratio	2.29 ± 0.20	2.04 ± 0.14

[a-b] Means within a row with no common superscript differ significantly ($P<0.05$).
[1] AL = *ad libitum* fed; RF = restricted fed.
[2] The 32 RF birds supply the RF and FINC feeding treatments with 16 birds each.
[3] Egg production traits for the 18 wk period ending at 59 wk of age.

Table 2-4. Body weight, feed intake, abdominal fatpad, and liver measurements at processing in long or short-term *ad libitum* fed or restricted fed broiler breeders with blank or estradiol-17β silastic implants at 59 wk of age

				Abdominal fatpad		Liver		
Source	BW	BW difference[1]	Daily feed intake[2]	Weight	Percentage[3]	Weight	Percentage[3]	Liver lipid content[4]
	(g)			(g)	(%)	(g)	(%)	(%)
Feed[5]								
AL	4142^a	33.2^b	140.2^b	306.2^a	7.39^a	75.2^b	1.81^b	34.9^b
RF	3640^b	6.9^b	128.0^b	196.4^b	5.26^b	65.1^b	1.80^b	34.1^b
FINC	3744^b	199.4^a	214.4^a	176.9^b	4.68^b	100.9^a	2.70^a	58.2^a
SEM	74	16.7	6.6	22.1	0.51	3.7	0.09	3.1
Hormone[6]								
BL	3851	87.3	168.6^a	229	5.79	81.2	2.13	41.2
E2	3833	72.0	153.1^b	224	5.77	79.6	2.08	43.5
SEM	60	13.6	5.2	18	0.43	3.1	0.08	2.6
Interaction								
AL-BL	4192	47.0	138.5	311.7	7.43	73.7	1.75	33.7
AL-E2	4091	19.4	141.9	300.6	7.35	76.7	1.87	36.1
RF-BL	3646	4.7	128.0	216.1	5.69	63.5	1.75	28.6
RF-E2	3633	7.9	128.0	176.8	4.83	66.6	1.84	39.6
FINC-BL	3714	210.3	222.1	159.2	4.23	106.3	2.88	61.4
FINC-E2	3774	188.6	201.1	194.6	5.12	95.5	2.53	54.9
SEM	103	23.6	9.4	31.0	0.73	5.2	0.13	4.5
				Probability				
Source of variation								
Feed	0.0001	0.0001	0.0001	0.0004	0.0014	0.0001	0.0001	0.0001
Hormone	0.83	0.43	0.050	0.85	0.98	0.72	0.67	0.53
Feed X hormone	0.75	0.79	0.014	0.48	0.49	0.32	0.16	0.16

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] BW difference = weight change during implantation period (4 d).

[2] Daily feed intake during the 4 d experimental period.

[3] Percentage = tissue weight / BW X 100.

[4] Lipid percentage = liver lipid weight / liver weight X 100.

[5] AL = *ad libitum* fed; RF = restricted fed; FINC = previously RF birds increased to AL feeding level.

[6] BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 2-5. Oviduct, ovary, and stroma weights at processing in long or short-term *ad libitum* fed or restricted fed broiler breeders with blank or estradiol-17β silastic implants at 59 wk of age

Source	Oviduct		Ovary		Initial stroma[2]		Bare stroma[3]	
	Weight	Percentage[1]	Weight	Percentage	Weight	Percentage	Weight	Percentage
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Feed[4]								
AL	67.2	1.62	72.4^{a}	1.74	9.48^{a}	0.229	5.83^{a}	0.141^{b}
RF	68.2	1.90	61.9^{b}	1.73	8.20^{b}	0.227	4.90^{b}	0.135^{b}
FINC	73.1	1.96	67.1^{ab}	1.80	9.76^{a}	0.263	5.97^{a}	0.160^{a}
SEM	3.3	0.10	2.9	0.09	0.44	0.012	0.26	0.007
Hormone[5]								
BL	73.5^{a}	1.94	64.0	1.68	9.29	0.244	5.66	0.148
E2	65.5^{b}	1.71	70.3	1.83	9.00	0.235	5.47	0.143
SEM	2.7	0.08	2.4	0.07	0.36	0.010	0.21	0.005
Interaction								
AL-BL	68.6	1.64	69.9	1.68	9.24	0.220	5.73	0.136
AL-E2	65.9	1.60	74.8	1.80	9.71	0.237	5.93	0.145
RF-BL	73.5	2.04	58.0	1.62	8.26	0.229	4.96	0.137
RF-E2	62.9	1.75	65.8	1.84	8.14	0.225	4.84	0.134
FINC-BL	78.4	2.12	64.0	1.74	10.36	0.282	6.30	0.171
FINC-E2	67.9	1.79	70.2	1.86	9.16	0.243	5.64	0.149
SEM	4.6	0.14	4.1	0.12	0.62	0.016	0.36	0.009
	Probability							
Source of variation								
Feed	0.40	0.060	0.042	0.81	0.035	0.066	0.011	0.032
Hormone	0.043	0.064	0.063	0.14	0.58	0.51	0.52	0.48
Feed X hormone	0.64	0.56	0.94	0.90	0.42	0.25	0.51	0.29

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Percentage = tissue weight / BW X 100.

[2] Initial stroma = ovary without the large yellow follicles (follicles >10mm diameter removed).

[3] Bare stroma = initial stroma without the small yellow follicles or large white follicles (follicles >2mm diameter removed).

[4] AL = *ad libitum* fed; RF = restricted fed; FINC = previously RF birds increased to AL feeding level.

[5] BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 2-6. Ovarian follicle numbers, incidence of follicular atresia, and mean large yellow follicle weight at processing in long or short-term *ad libitum* fed or restricted fed broiler breeders with blank or estradiol-17β silastic implants at 59 wk of age

Source	Ovarian follicles[1]				
	LYF number	SYF number	LWF number	Incidence of follicular atresia[2]	Mean LYF weight
	(#)			(%)	(g)
Feed[3]					
AL	5.92	9.59	35.0	39.3	10.56
RF	5.68	8.06	28.3	39.3	10.35
FINC	5.71	9.45	33.0	18.8	10.03
SEM	0.20	0.89	2.2	12.0	0.51
Hormone[4]					
BL	5.79	9.49	31.6	19.0	9.84^{b}
E2	5.75	8.58	32.5	45.8	10.79^{a}
SEM	0.16	0.72	1.8	9.8	0.40
Interaction					
AL-BL	6.00	10.57	32.4	28.6	10.04
AL-E2	5.83	8.63	37.5	50.0	11.09
RF-BL	5.50	7.75	28.1	28.6	9.85
RF-E2	5.86	8.38	28.4	50.0	10.82
FINC-BL	5.86	10.14	34.3	0.0	9.62
FINC-E2	5.57	8.75	31.6	37.5	10.45
SEM	0.28	1.24	3.1	16.7	0.69
	Probability				
Source of variation					
Feed	0.68	0.40	0.082	0.36	0.61
Hormone	0.89	0.38	0.73	0.059	0.029
Feed X hormone	0.48	0.56	0.47	0.86	0.90

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] LYF = large yellow follicles (>10mm diameter); SYF = small yellow follicles (5-10mm diameter); LWF = large white follicles (2-5mm diameter).

[2] Percentage of birds having incidence of atretic follicles > 5 mm in diameter.

[3] AL = *ad libitum* fed; RF = restricted fed; FINC = previously RF birds increased to AL feeding level.

[4] BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 2-7. Distribution of ingested radiolabel between the liver, ovary, and abdominal fatpad at processing in long or short-term *ad libitum* fed or restricted fed broiler breeders with blank or estradiol-17β silastic implants at 59 wk of age

	Deposition of radioactivity[1]		
Source	Liver	Ovary	Abdominal fatpad
		(%)	
Feed[2]			
AL	17.8^{b}	48.2^{a}	34.0
RF	13.6^{b}	51.8^{a}	34.6
FINC	36.3^{a}	26.2^{b}	37.5
SEM	2.6	4.0	3.5
Hormone[3]			
BL	20.7	41.9	37.4
E2	24.4	42.2	33.3
SEM	2.1	3.3	2.8
Interaction			
AL-BL	12.5^{b}	50.7	36.8
AL-E2	23.1^{a}	45.6	31.3
RF-BL	10.3	48.9	40.8
RF-E2	16.9	54.7	28.4
FINC-BL	39.4	25.9	34.7
FINC-E2	33.3	26.4	40.3
SEM	3.7	5.6	4.9
		Probability	
Source of variation			
Feed	0.0001	0.0001	0.65
Hormone	0.54	0.85	0.50
Feed X hormone	0.042	0.54	0.85

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Deposition calculated as a percentage of total measured ^{14}C activity in the liver, the ovary (including ovulated follicles), and the abdominal fatpad.

[2] AL = *ad libitum* fed; RF = restricted fed; FINC = previously RF birds increased to AL feeding level.

[3] BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 2-8. Plasma estradiol-17β concentration, plasma lipid content, and very low density lipoprotein lipid content, triglyceride (TG) and phospholipid (PL) content, and TG:PL ratio at processing in long or short-term *ad libitum* fed or restricted fed broiler breeders with blank or estradiol-17β silastic implants at 59 wk of age

Source	Estradiol-17β concentration	Plasma lipid concentration	VLDL traits Lipid concentration	VLDL traits TG content	VLDL traits PL content	VLDL traits TG:PL ratio
	(pg/mL)	(mg/mL)	(mg/mL)	(mg/mL)	(mg/mL)	
Feed[1]						
AL	195.9	31.7	22.7	15.68	5.22	3.08
RF	194.6	24.1	18.0	11.94	5.01	2.52
FINC	231.4	32.1	25.0	17.21	6.00	2.90
SEM	14.2	3.3	2.9	2.15	0.69	0.21
Hormone[2]						
BL	156.7^b	29.1	21.9	15.28	5.14	2.98
E2	257.9^a	29.5	21.9	14.60	5.69	2.68
SEM	11.6	2.7	2.3	1.72	0.56	0.18
Interaction						
AL-BL	150.3^b	27.9	20.4	14.39	4.70	3.24
AL-E2	241.5^a	35.5	24.9	16.98	5.74	2.92
RF-BL	144.9^b	22.1	16.0	10.86	4.29	2.61
RF-E2	244.2^a	26.0	20.0	13.01	5.74	2.43
FINC-BL	175.0^b	37.2	29.3	20.60	6.42	3.10
FINC-E2	287.9^a	27.0	20.8	13.81	5.58	2.70
SEM	19.8	4.6	4.0	3.04	0.98	0.30
	Probability	Probability	Probability	Probability	Probability	Probability
Source of variation						
Feed	0.12	0.16	0.22	0.19	0.54	0.20
Hormone	0.0001	0.91	0.99	0.78	0.48	0.24
Feed X hormone	0.86	0.14	0.20	0.20	0.43	0.94

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] AL = *ad libitum* fed; RF = restricted fed; FINC = previously RF birds increased to AL feeding level.

[2] BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 2-9. Differences between processing (Day 4) and initial (Day 0) values for plasma estradiol-17β concentration, plasma lipid content, and very low density lipoprotein lipid content and triglyceride:phospholipid (TG:PL) ratio in long or short-term *ad libitum* fed or restricted fed broiler breeders with blank or estradiol-17β silastic implants at 59 wk of age

			VLDL trait differences	
Source	Estradiol-17β concentration difference[1]	Plasma lipid concentration difference	Lipid concentration difference	TG:PL ratio difference
	(pg/mL)	(mg/mL)	(mg/mL)	
Feed[2]				
AL	44.7	8.6	4.8	0.91
RF	61.3	3.9	3.0	0.57
FINC	73.4	13.9	11.1	0.77
SEM	17.4	2.7	2.9	0.33
Hormone[3]				
BL	2.6^{b}	7.4	5.5	0.90
E2	117.3^{a}	9.7	7.1	0.61
SEM	14.2	2.2	2.4	0.26
Interaction				
AL-BL	-5.4^{b}	7.2	3.9	1.23
AL-E2	94.8^{a}	10.0	5.6	0.58
RF-BL	-10.5^{b}	-0.3	-0.6	0.51
RF-E2	133.8^{a}	8.1	6.6	0.63
FINC-BL	23.5^{b}	15.2	13.0	0.95
FINC-E2	123.3^{a}	10.9	9.1	0.61
SEM	24.2	3.9	4.2	0.46
	Probability	Probability	Probability	Probability
Source of variation				
Feed	0.57	0.061	0.13	0.77
Hormone	0.0001	0.48	0.63	0.44
Feed X hormone	0.59	0.26	0.41	0.70

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Variable difference = day 4 value - day 0 value.

[2] AL = *ad libitum* fed; RF = restricted fed; FINC = previously RF birds increased to AL feeding level.

[3] BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 2-10. Distribution of ingested radiolabel between the TG and PL lipid classes of the plasma VLDL fraction at processing in long or short-term *ad libitum* fed or restricted fed broiler breeders with blank or estradiol-17β silastic implants at 59 wk of age

	VLDL radioactivity[1]		
Source	TG	PL	TG:PL ratio
	(%)		
Feed[2]			
AL	76.6^{a}	23.4^{b}	3.38^{a}
RF	71.6^{b}	28.4^{a}	2.69^{b}
FINC	76.7^{a}	23.3^{b}	3.63^{a}
SEM	1.4	1.4	0.24
Hormone[3]			
BL	75.5	24.5	3.33
E2	74.3	25.7	3.14
SEM	1.2	1.2	0.19
Interaction			
AL-BL	77.5	22.5	3.46
AL-E2	75.7	24.3	3.30
RF-BL	68.6	31.4	2.29
RF-E2	74.5	25.5	3.09
FINC-BL	80.5^{a}	19.5^{b}	4.42^{a}
FINC-E2	72.8^{b}	27.2^{a}	3.03^{b}
SEM	2.1	2.1	0.32
	Probability		
Source of variation			
Feed	0.049	0.049	0.045
Hormone	0.48	0.48	0.47
Feed X hormone	0.033	0.033	0.041

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] VLDL activity calculated as percentage of total measured ^{14}C activity in the TG and PL lipid classes of the VLDL. Activity in other lipid classes was negligable.

[2] AL = *ad libitum* fed; RF = restricted fed; FINC = previously RF birds increased to AL feeding level.

[3] BL = blank silastic implant; E2 = estradiol-17β silastic implant.

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3. EFFECTS OF FEEDING REGIMEN AND TIMING OF SEXUAL MATURITY ON LIPID ALLOCATION, OVARIAN MORPHOLOGY, AND PLASMA LIPID TRAITS IN BROILER BREEDER HENS

3.1 INTRODUCTION

Broiler breeder pullets are routinely feed restricted from an early age to circumvent reproductive problems brought about by selection for growth. Excessive BW and lipid content in broiler breeder hens is negatively associated with settable egg production, fertility, hatchability, and egg shell quality (Yu *et al.*, 1992; Robinson *et al.*, 1993a). This effect is primarily the result of excess large yellow yolky follicle production, which is associated with double hierarchies and multiple ovulations (Hocking *et al.*, 1989; Yu *et al.*, 1992). Increased feed allocation causes excess large yellow follicle (LYF) numbers at sexual maturity (SM) (first oviposition). Increased LYF numbers are associated with increased production of unsettable eggs (Jaap and Muir, 1968), whereas reduced LYF numbers through feed restriction are associated with a subsequent increased rate of lay (Hocking *et al.*, 1987; Yu *et al.*, 1992). Full-fed broiler breeders can have an average of 12 LYF on the ovary at SM compared with 7 LYF observed in restricted fed birds (Hocking *et al.*, 1987; Yu *et al.*, 1992).

A sudden increase in feeding level is reported to result in a disproportionately high accumulation of carcass fat, increased LYF weights, and an increased incidence of double hierarchies of LYF (Robinson *et al.*, 1993b). Increasing feed access to *ad libitum* levels was believed to relate to a loss of regulation of the ovarian hierarchy within a few days of overfeeding (Robinson and Wilson, 1996). Early maturing birds can also have increased LYF numbers (Hocking *et al.*, 1988) and multiple hierarchies (Hocking, 1992; Renema *et al.*, 1997a). In the birds of Robinson *et al.* (1995), a one follicle increase in birds on an accelerated feeding regimen during sexual maturity was associated with a 10 egg reduction in output. The state of the ovary at SM appears to set the tone for reproductive performance for the duration of the breeder period. The identification of a plasma hormone or lipid parameter that is a good indicator of ovary morphology at or prior to the onset of lay would be of value to the poultry breeder industry because it would allow early selection of birds with the best potential egg production.

The following experiment examined the effects of feeding regimen between photostimulation and SM on lipid allocation to the liver, abdominal fatpad, and ovary, on ovarian morphology, and on plasma lipid traits. The slow-feed (SF) and fast-feed (FF) feeding regimens of Robinson *et al.* (1995) were used because they have previously altered LYF number at SM. The relationship between timing of SM (early or late maturing) in each feeding regimen with ovary morphology and plasma lipid parameters were examined.

3.2 MATERIALS AND METHODS

Stocks and Management

A total of 100 day old Shaver Starbro[1] broiler breeder pullets were reared in a 4.75 m X 5.85 m floor pen in a light-tight facility. Birds were allowed *ad libitum* access to water and grown to a breeder recommended target BW curve. Birds were fed a starter diet from 0 to 3 wk of age, a grower diet from 3 to 21 wk of age, and a breeder diet from 21 wk of age until processing at SM (first oviposition) (Table 3-1). All diets were fed in a mash form. Following *ad libitum* feeding to 2 wk of age, Skip-a-day feeding was used for the duration of the rearing period. Daily restricted feeding was used during the breeder period. Body weight was monitored in individual birds at 4 wk intervals and with group weights during all other weeks. Pullets received 24 h of light (24L:0D) for the first 24 h, which was decreased to 8 h of light per d (8L:16D) until photostimulation (PS).

At 21 wk of age, birds were individually weighed and the 58 birds closest to the pen mean selected. Ten birds selected for the PS (photostimulation) group were processed immediately. These birds served as a starting control group and were used to assess the degree of reproductive development at PS. Birds were euthanatized by cervical dislocation. The breast muscle, liver, abdominal fatpad, oviduct, and ovary were dissected and weighed. The remaining 48 birds were randomly assigned to one of two feeding regimens as follows: FF (fast feed) received an abrupt, generous feed increase at PS (21 wk of age); and SF (slow feed) received moderate increases in feed allocation between 21 wk of age and SM (Table 3-2). For SF birds, increases of 5 g or more were divided into two smaller increases per wk. Birds of the FF and SF feeding regimens had similar BW targets at SM (25 to 27 wk of age). Birds were individually caged at 20 wk of age and feeding treatments commenced at 21 wk of age. Day length was increased to 11L:13D, followed by a 1 h increase in light per week to 15L:9D. Individual BW was recorded at weekly intervals.

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[1] Shaver Poultry Breeding Farms Ltd., Box 400, Cambridge, ON, N1R 5V9

Birds were divided into one of two 'day of SM' groups at first oviposition to examine time effects of maturation date on ovary morphology and lipid traits. The first 50% of birds to lay an egg in either the FF or SF treatments were assigned to the EARLY SM group and the last 50% of birds to lay were assigned to the LATE SM group. This resulted in the 2 X 2 factorial design with the main effects, Feed and SM Time interacting to form the treatments: FF-EARLY, FF-LATE, SF-EARLY, and SF-LATE. The experimental protocol was approved by the Animal Policy and Welfare Committee of the Faculty of Agriculture, Forestry and Home Economics of the University of Alberta.

Experimental Procedures

Lipid Partitioning: Birds were weighed and an initial 10 cc blood sample was taken for plasma lipid and hormone analysis prior to initiation of feeding treatments at 21 wk of age. Partitioning of lipid to major storage sites was traced using radiolabeled palmitate. At 2 d intervals beginning at PS (21 wk of age), birds were given 0.188 μCi of 1-[^{14}C] palmitate[2] in 300 μL of canola oil in a gelatin capsule[3]. Approximately 3 μCi of radiolabeled lipid was anticipated to be administered by expected day of SM.

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[2] SA = 54 mCi/mmole, NEN Research Products, Boston, MA.

[3] Size No. 1 Empty gelatin capsules, T.U.B. Enterprises, North Augusta, ON

Birds were maintained on assigned feeding regimens until first oviposition. At this time BW was recorded and, the following morning at 0800 h, a final 10 cc blood sample was taken for analysis of plasma traits as above. Body weight was recorded, birds were euthanatized and the breast muscle, liver, abdominal fatpad, oviduct, and ovary were removed and weighed. The liver and abdominal fatpad were then stored at -15°C until radioactivity was quantified. The number and weight of large yellow ovarian follicles (LYF) (>10 mm diameter), and the number of small yellow follicles (SYF) (5-10 mm diameter), large white follicles (LWF) (3-5 mm diameter), and medium white follicles (MWF) (1-3 mm diameter) were recorded. Follicle size classifications were based on previous reports (Robinson and Etches, 1986) with the following changes: the LWF size range was continuous with the SYF; and the term, MWF, was introduced to describe follicles 1-3 mm in diameter. Stroma weight was recorded with the LYF removed (initial stroma) and again when the SYF, LWF, and MWF had also been removed (bare stroma) to assess treatment effects on large and small follicle types separately. An assessment of the potential for multiple ovulations to occur was determined by assigning LYF of similar size (differing by less than 1 g or 1 mm diameter) to the same position in the hierarchy as reported previously (Renema *et al.*, 1995). Total number of positions and proportion of follicles in a multiple hierarchical arrangement were recorded. Number of complete hierarchies of LYF on the ovary was calculated by dividing LYF number by the number of positions in the hierarchy. The incidence of internal ovulation (as evidenced by yolk residue in the body cavity) was assessed. Follicles with a discolored and/or shrunken appearance were considered atretic (Gilbert *et al.*, 1983). The incidence of atresia of the yellow follicles (> 5 mm diameter) and of the small follicle (< 5 mm diameter) was assessed by counting all visible atretic follicles in the appropriate ranges. Following assessment of ovarian morphology, all parts of the ovary were recombined. Egg yolks from eggs laid during the 72 h following ingestion of the radiolabeled lipid as well as developing eggs found in the oviduct at processing were isolated and homogenized with the ovary. Ovaries were frozen, and all previously frozen tissue samples freeze-dried for 7 d.

Liver, abdominal fatpad, and the combined ovary and yolk lipids were assessed using standard chemical analysis procedures (AOAC, 1980) on duplicate 1 g (abdominal fatpad) or 2 g (liver and ovary) samples. True tissue lipid content was calculated by adjusting recorded values to account for moisture loss during the tissue drying process. Isolated tissue lipid was dissolved with 3 mL of petroleum ether following final weighing and an 800 µL sub-sample placed in a pre-weighed scintillation vial. Petroleum ether was evaporated off and sub-sample weight determined. Ten mL of a toluene-based organic scintillation solution (Toluene with 4.0 g/L PPO and 0.1 g/L POPOP) was added to all vials and liquid scintillation counting (LSC) performed with a Packard 1600 CA Tri-Carb Liquid Scintillation Analyzer using a 5 min counting time. Color quenching of tissue samples was corrected for using the previously calculated efficiencies of 89%, 96%, and 93% for liver, abdominal fatpad and ovarian tissue counts, respectively (Renema *et al.*, 1997c). Radioactivity incorporated into liver, abdominal fatpad, and ovary tissue was expressed as a percentage of the total radioactivity isolated.

Blood Plasma Analysis: Blood samples were taken from the brachial vein using EDTA-coated vacuum blood collection tubes with 21 gauge needles. Blood was centrifuged at 1500 *g* for 20 min at 3°C. A preservative solution of streptomycin sulfate and penicillin-G (50 U each/mL stock) was added to plasma at a level of 20 µL/mL to limit bacterial and mold growth. Plasma for subsequent lipid quantification was stored at -30°C. Plasma undergoing VLDL lipid quantification and analysis was further treated with 1 µL/mL of a 1M benzamidine solution and 5 µL/mL of a 0.2M phenylmethylsulfonyflouride (PMSF) solution to limit protease activity. Two mL of plasma were overlayered with 2 mL of 0.85% KBr solution (density = 1.0063) and ultracentrifuged in a Beckman 50 Ti rotor at 40,000 rpm for 7 hr at 20°C. The VLDL layer was removed with the top half of the tube contents as previously described (Renema *et al*, 1997c).

Total plasma lipid weight and VLDL lipid weight were determined by Folch lipid extraction. Extracted samples were dried to constant weight under a nitrogen stream with gentle heating. The lipid classes of the VLDL lipids were separated and quantified by IATROSCAN

(TLC/FID)[4] as described by Renema *et al.* (1997c), using a benzene: chloroform: acetic acid: methanol (88.9:10.5:3.9:2.6 vol/vol) developing solvent system.

Plasma estradiol-17β concentration was determined using a RIA[5] kit as described by Renema *et al.* (1997c). All samples were analyzed in a single RIA with an intra-assay coefficient of variation of 5.72%.

Statistical Analysis

The variance of data recorded from birds processed at PS is presented as the standard error of the mean for each parameter. Feeding regimens were applied to cages in a completely randomized design. The experiment was a 2X2 factorial design with the main effects, Feed and SM Time. Sexual maturity data were evaluated by two-way analyses of variance using the General Linear Models (GLM) procedures of SAS® (SAS Institute, 1994). Source of variation for parameters measured in birds processed at PS was variation between birds. Sources of variation for initial and final plasma traits, and for carcass parameters were feeding regimen, SM time, and the interaction feeding regimen X SM time. Differences between means were evaluated with Fisher's protected LSD procedure (Peterson, 1985). The error variation for all variables consisted of the variation between birds within the interaction.

Pearson correlation coefficients (Steel and Torrie, 1980) were computed between reproductive, carcass, and plasma parameters across all treatments and within each feeding regimen and time group. Unless otherwise stated, all statements of significance were based on testing at the $P < 0.05$ level.

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[4] Iatron Laboratories, Inc., Tokyo, 101 Japan

[5] Kit Number TKE25, Diagnostic Products Corp., Los Angeles, CA 90045-5597

3.3 RESULTS AND DISCUSSION

Carcass Morphology at Photostimulation (20 wk of age)

Carcass parameters of birds processed at PS are presented in Table 3-3. With a BW of 1939 g and a shank length of 102.2 mm, these birds were very similar to those of a previous trial by Goerzen (1996), and had a shorter shank length and BW than those of Robinson *et al.* (1996). Abdominal fatpad weight was about half that reported by Robinson *et al.* (1996) and 23% of the value reported by Fattori *et al.* (1993) at a similar age. No LYF were present on the ovary at PS, and reproductive tract weights at this time were similar to previously reported values (Fattori *et al.*, 1993; Robinson *et al.*, 1996). Immature ovary weight correlated well with BW on both an absolute (r=0.843; P=0.002) and a relative basis (r=0.788; P=0.007), indicating that ovaries in the heaviest birds may be more developed at this time.

Carcass Morphology at Sexual Maturity

The FF birds entered lay at a higher BW (2733 g) and at a younger age (167.9 d) than the SF birds (2618g, 171.3 d) (Tables 3-4 3-5). However, by the time the birds were processed 12 to 24 h after laying their egg, BW were similar (2622 g and 2580 g for FF and SF birds, respectively). The BW growth curve from PS to SM is presented in Figure 3-1, in which there were significant differences in BW between FF and SF birds at 23 and 24 wk of age. After this time the standard error increased due to bird numbers decreasing as they were processed at SM. When evaluated based on time of SM, LATE birds came into lay 9% heavier (Table 3-4) and 10.6 d later than the EARLY birds (Table 3-5). The interval between EARLY and LATE birds reaching SM was greater in the SF than in the FF birds (13.4 *vs.* 6.9 d, respectively), indicating the nutritional advantage of the FF regimen primarily affected the slower maturing birds. An additive plot of birds reaching SM for the FF and SF feeding regimens indicated that except for a delay in SF curve between 17 and 23 d after PS, the curves are very similar (Figure 3-2). The extra feed allocation to FF birds appears to have allowed them to reach their BW or body composition threshold for reproduction (Brody *et al.*, 1980) in 3.4 fewer days.

With the exception of day of SM, there were no significant differences for carcass tissue and organ weights for the FF and SF feeding regimens (Tables 3-4 and 3-5), indicating that the only discernible effect of the FF feeding regimens was to advance SM by 3.4 d. Previous application of the FF and SF feeding regimens resulted in an increased ovary weight and a 1.0 follicle increase in LYF in FF birds (Robinson *et al.*, 1995). The ovary weights reported by Robinson *et al.* (1995) were 6.4 g greater in FF than SF birds, compared to a 2.9 g non-significant difference in the current study. The mean time from PS to SM in the current experiment was 22.6 d (Table 3-5), which is 1 wk shorter than that reported by Robinson *et al.* (1995, 1996), and 12 d shorter than that reported by Goerzen (1996) for similarly managed birds. The BW at SM was similar in all of these experiments, indicating that BW may be more important than age in the control of sexual maturation in the strain used in these experiments and that BW at PS may have been greater in the current study than those of the other studies.

Abdominal fatpad weight was 48.7 and 47.5 g in the FF and SF treatment birds, respectively (Table 3-4), representing 1.84% of bird BW. This is about half that reported by Robinson *et al.* (1995) and lower than the 2.3% reported by Robinson *et al.* (1996) for similar birds. Although abdominal fatpad weight was also very low at PS (Table 3-3), the low weight at SM is likely due to the low age of SM in this study. Abdominal fatpad weight changed significantly with time of SM, with weights of 41.1 g and 54.5 g in EARLY and LATE birds, respectively (Table 3-4). The weight of breast muscle was greater in LATE than EARLY maturing birds (Table 3-4). This difference was driven by the interaction of the SF feeding regimen with time of SM, with breast muscle weights of 396 g *vs.* 448 g recorded for SF-EARLY and SF-LATE birds, respectively. Increased breast muscle weight was a function of increased BW ($r=0.707$; $P<0.0001$) (Table 3-4). Relative breast muscle weight was greater in the EARLY birds, however, which had a lower BW at SM than the late birds. The first birds to reach SM likely had better 'fleshing' at the time relative to the remaining birds, as demonstrated in the higher proportion of breast muscle in the EARLY birds. The reduction in breast muscle proportion in LATE birds is accounted for in part by the increased proportion of abdominal fatpad (Table 3-4), but may also indicate that part of the reason LATE birds required more time to achieve SM was due to their inferior fleshing.

Reproductive Morphology at Sexual Maturity

Absolute oviduct weight was the greatest in birds reaching SM later (Table 3-5). The LATE SM group oviducts weighed 13.3% more than the EARLY group oviducts. However, this difference was not present on a relative basis, demonstrating the difference may be due to the increased BW of the LATE birds. The overall correlation between oviduct weight and BW was significant (r=0.505; P=0.0004), although the relationship only approached significance in the LATE birds (P=0.07).

Ovary weight did not differ due to feeding regimen or time effects (Table 3-5). Ovary weight was correlated with BW overall (r=0.459; P=0.002), but this relationship was primarily in EARLY birds (P=0.006 *vs.* P=0.17 in EARLY and LATE birds, respectively). In EARLY birds, ovary weight increased both with later time of SM (P=0.0007) and with increased BW, whereas the ovaries of LATE birds were not influenced by SM or BW.

Large yellow follicle number was not affected by feeding program or timing of SM (Table 3-6). Body weight and lean body mass have been reported to have the most influence on LYF number, whereas lipid content after correcting for BW is less important (Hocking *et al.*, 1989; Hocking, 1993, 1996). In the current experiment, the LYF number of the slightly heavier FF feeding regimen birds were 8.50 compared to 8.17 in SF birds. Contrary to these reports of the lack of association between body fat and follicle numbers, however, abdominal fatpad weight was positively correlated with LYF number (r=0.316; P=0.031). Although it is likely that this effect is associated with changes in BW and fat content of birds with increasing day of SM, it demonstrates that energy stores may have some influence on the bird's ability to construct a LYF hierarchy. The number of LYF in SF birds was higher than reported values for similarly treated birds at SM (Hocking, 1993; Goerzen, 1996; Robinson *et al.*, 1996) by 1 to 1.8 follicles. The rate of reproductive development in SF birds was similar to that of the FF birds and both appeared to have been accelerated relative to birds in other published reports.

Ovarian stroma weight was not affected by feeding program, but was significantly increased in time (Table 3-5). Although the 37% increase in initial stroma weight could be due to increased small follicle numbers, the 22% difference remaining in the bare stroma weight (all follicles > 1mm diameter removed) suggests that the stromal mass has increased, and possibly the follicle carrying capacity with it. Small follicle numbers increased in time, with the SYF, LWF, and MWF numbers of LATE birds being 51%, 39%, and 80% greater, respectively, than those of EARLY birds (Table 3-6). The bare stroma supported more MWF with a later time of SM (P=0.002), with the EARLY and LATE bird stromas maintaining populations of 55.2 and 83.6 MWF per g of stroma, respectively. The population of white follicles was not related to LYF numbers in either EARLY or LATE birds, concurring with the observations of Hocking *et al.* (1989). Hocking (1996) stated that the probability of recruitment to the LYF hierarchy may not be closely associated with the growth of pre-hierarchical follicles and that these processes may be related to different underlying physiological systems.

Associated with an increased number of small follicles with delayed SM came an increased number of small atretic follicles (<5mm diameter). The overall small atretic follicle number was significantly correlated with day of first egg (r=0.474; P=0.0007). Small atretic numbers for LATE birds were only numerically increased over EARLY numbers, however (P=0.12). Unlike LYF and MWF numbers, which were only correlated to day of first egg in the EARLY birds, small atretic number was only correlated with day of first egg in the LATE birds (r=0.480; P=0.015 in LATE *vs.* r=0.398; P=0.60 in EARLY).

The mean LYF weight and number of hierarchy positions did not differ in time or with feeding regimen (Table 3-6). There were an average of 1.49 complete hierarchies of LYF due to the presence of 5.92 positions on the ovary to accommodate 8.34 LYF on average. Hocking (1993) stated that higher food intakes in birds of similar BW result in increased LYF numbers. Birds of the current study had statistically similar BW at processing when compared by feeding regimen, and both groups consumed 2578 g of feed on average between PS and SM.

Lipid Allocation

The proportion of radiolabel deposited into the liver, ovary, and abdominal fatpad was approximately 18.0, 35.5, and 46.5% of recovered activity, respectively (Table 3-7). Values recorded for the FF and SF feeding regimens differed by less than 1.1% in each tissue, indicating how similar these two populations were when they reached SM. In the LATE maturing birds, the proportion of activity allocated to the ovary was reduced to 31.1% from 39.4% in EARLY birds. Activity was diverted to the abdominal fatpad, where proportion of activity increased from 41.1% in EARLY birds to 52.0% in LATE birds. This shift is indicative of the continually increasing abdominal fatpad weight in birds reaching SM later (Table 3-4). Particularly in the period directly after PS, the slower development of the ovary in LATE birds will allow a greater proportion of available radiolabel to be allocated to the abdominal fatpad. A high proportion of activity may have also gone to the abdominal fatpad to meet the growth requirements at this age. In 59 wk old broiler breeders, Renema *et al.* (1997c) reported only 34.6% of activity being in the abdominal fatpad of restricted birds, whereas 52% was found in the ovary. In broiler lines selected for high or low plasma VLDL concentration, Griffin *et al.* (1989) found that the fatter, high-VLDL birds deposited radiolabeled VLDL into the abdominal fatpad at a rate over 2.5-fold greater than that of the low-VLDL birds. In the current experiment, both abdominal fatpad activity deposition and plasma VLDL concentration were increased in LATE SM birds. Bacon *et al.* (1978) injected radiolabeled VLDL into laying hens and after 6 h recovered 20%, 30%, and 25% of injected activity in the liver, ovary, and carcass, respectively. The remainder of the activity was presumably oxidized to CO_2. Total radioactivity recovered in the liver, abdominal fatpad, and ovary represents 25.8% (SF) to 26.3% (FF) of radioactivity administered in the gel capsules. Recovery was considerably better in the 72 hr radioactive lipid allocation period of Renema *et al.* (1997c), where 51.2% of activity was recovered from restricted fed birds.

Plasma Hormone and Lipid Parameters

Plasma estradiol-17β concentration did not differ due to feeding regimen or to time of SM (Figure 3-8). It was, however, significantly greater in the PS measurement of the SF-EARLY birds (67.4 pg/mL) than in the SF-LATE birds (43.3 pg/mL), causing the variation in the SM Time values to approach significance (P=0.081). It is possible that the PS measurement of estradiol-17β concentration can be used to predict day of SM in birds which are slower than average for coming into lay. The difference in plasma estradiol-17β concentration between PS and first oviposition was correlated across all treatments with plasma VLDL at processing (P=0.009), processing BW (P=0.030), and most strongly with LYF number (P=0.0004). Plasma estradiol-17β concentration was negatively correlated with small follicle atresia (P=0.048). Higher hormone levels may be due in part to fewer of the estrogen producing white follicles becoming atretic.

Plasma lipid parameters did not differ with feeding regimen (Table 3-8). Time of SM, however, was associated with plasma VLDL lipid content differences at both PS and at SM. The initial VLDL concentrations were the greatest in the EARLY birds (1.52 mg/mL) compared to the LATE birds (1.16 mg/mL), which may indicate the EARLY birds had already undergone some initial sexual development by this time. The PS plasma VLDL lipid concentration were negatively correlated with ovary weight at SM (r=-0.350; P=0.016) and with all follicle number classes (LYF: r=-0.320, P=0.028; SYF: r=-0.317, P=0.028; LWF: r=-0.331, P=0.0214; and MWF: r=-.396, P=0.0053). Plasma VLDL lipid concentration increased 11.8-fold between PS (1.35 mg/mL) and SM (14.81 mg/mL) whereas total plasma lipid concentration underwent only a 3.3-fold increase. The large increase in plasma VLDL lipids relative to plasma lipids as birds approached SM is in accordance with the observations of Yu *et al.* (1976). At SM the plasma VLDL concentration in EARLY birds was 12.95 mg/mL compared with 16.68 mg/mL in LATE birds. This difference may be associated with the observed increased rate of lipid allocation to the abdominal fatpad and abdominal fatpad weight.

Affectors of LYF Number

The influence of BW, carcass, and plasma lipid parameters on LYF number can be altered in time. Although LYF numbers across treatments were correlated with BW (r=0.448; P=0.002), this may be confounded by day of SM. Birds maturing early can have elevated LYF numbers (Hocking *et al.*, 1988), which will tend to be arranged in multiple hierarchies (Hocking, 1992; Renema *et al.*, 1997a). Day of first egg was highly correlated with BW (r=0.623; P<0.0001) but not with LYF number (P=0.18). Despite significant increases in BW based on time of SM (Table 3-4), LYF numbers did not increase at the same rate. The 5.7% numerical increase in LYF number was considerably lower than the 39% to 80% significant increases observed within small follicle classes (Table 3-6).

The modulator of LYF appears to be the rate of small follicle atresia. In EARLY birds, where LYF number is primarily influenced by BW, the relationship between small follicle atresia and LYF number was poor (P=0.59). In LATE birds, where BW was not significantly related to LYF number (P=0.16), increased small follicle atresia was correlated with reduced LYF number (r=-0.586; P=0.002). Hocking *et al.* (1987) also observed an inverse relationship between atretic white follicles and LYF in hens of various ages. This relationship was not observed by Hocking *et al.* (1989) in birds at SM, however, and it was speculated that a stable population of white follicles had not yet developed at or near first egg. The results of the current experiment suggest that the relationship between small follicle atresia and LYF is firmly established in LATE birds, but that the establishment of the hierarchy of LYF in EARLY birds is affected by alternate cues for control of LYF recruitment.

Factors positively correlated with LYF number in EARLY birds were day of SM (P=0.0004), BW at SM (P=0.020), and initial (PS) plasma lipid concentration (P=0.023). There was a negative correlation with the proportion of liver lipids (P=0.028) and with the proportion of radiolabeled lipid being allocated to the liver (P=0.002). In EARLY birds, BW appeared to be associated with LYF number, as suggested by Hocking *et al.* (1989) and Hocking (1993, 1996). It is not clear why LYF numbers increase with day of SM in the EARLY period. It may be that the photostimulatory cue in the first birds to commence lay was less stimulatory as they likely had already undergone some sexual maturation by this time, resulting in fewer LYF at SM. Birds with a genetically lower BW threshold for SM may also come into lay with less external signaling. In a previous lipid allocation study in older birds, allocation of radiolabel to the liver was primarily at the expense of the ovary ($P<0.0001$). In the current study, liver radiolabel deposition was also at the expense of the ovary in the EARLY birds ($r=-0.449$; $P=0.036$). Along with the elevated initial plasma lipid concentration, this suggests that birds with increased LYF number are better able to clear lipid from the liver for deposition or use at other sites. The rate of lipid synthesis rather than mobilization is altered in a feed-restricted state (Leclercq, 1975), so it may be that the activities of liver enzymes involved in lipid synthesis differ in these birds. Plasma lipid concentration was not related to carcass fatness.

The LYF number of LATE birds was positively correlated with liver weight ($P=0.030$), plasma estradiol-17β concentration at SM ($P=0.005$), and with the proportion of radiolabeled lipid being allocated to the ovary ($P=0.005$). LATE bird LYF number was negatively correlated with small follicle atresia ($P=0.002$). These birds may have been delayed in reaching SM because they had not achieved an age, BW, or body composition threshold required for reproductive function (Brody *et al.*, 1980). The correlation of LYF with the liver, where yolk precursor VLDL is produced (Burley *et al.*, 1993), indicates that increased yolk deposition could be due in part to a positive state of energy balance emphasizing lipid synthesis. The role of estrogen in the formation of a yolk-specific population of VLDL particles has been well characterized (Walzem, 1996). Estrogen stimulates hepatic fatty acid and TG synthesis and formation of surface lipids to package the characteristically smaller VLDL particles destined for ovarian uptake (Walzem, 1996). The intimate link between plasma estradiol-17β concentration, liver weight, and lipid deposition to the ovary likely reflect the estrogen-stimulated production of ovary-specific VLDL. Renema *et al.* (1997b) reported that the ratio of triglycerides (TG) to phospholipids (PL) in the plasma VLDL fraction related to ovarian morphology. A low VLDL-TG:PL ratio was associated with increased ovary and stroma weights, whereas the proportional abdominal fatpad weight was reduced. Liver-synthesized VLDL for ovarian deposition has a smaller diameter than other VLDL (Griffin *et al.*, 1982) due to a reduced TG content (Walzem, 1996).

Although increased levels of small follicle atresia in LATE birds was not associated with increased small follicle number (P=0.65), it was clearly inversely related to the LYF complement. The major mechanism moderating egg production is believed to be either the control of atresia within white follicles, or the rate of recruitment to the hierarchy of yellow follicles (Hocking *et al.*, 1987). The current results demonstrate an important role for small follicle atresia in these mechanisms.

This experiment examined the effects of feeding regimen and timing of SM on carcass, ovarian, and plasma characteristics. Although feeding regimen had no significant affects in the current study, an accelerated feeding program would normally be expected to accentuate the differences observed for the time of SM effect. Birds would be expected to come into production quickly, and LYF number would be related primarily to BW at SM. A more gradual feeding program may generate more birds with delayed SM, in which LYF number would be related to plasma estradiol-17β concentration rather than to BW. This study indicated that the physiological systems controlling reproductive function and LYF recruitment continue to develop even as birds come into production. Accelerated or *ad libitum* type feeding programs may cause ovary development to proceed more quickly than the ovarian mechanisms controlling follicular recruitment are able to be established, which may contribute to excess LYF production. This study demonstrated that BW can be used as an indicator of LYF number in birds coming into production quickly and that plasma estradiol-17β concentration at SM may be a more appropriate predictor of LYF number for birds coming into production later.

TABLE 3-1. Composition and analysis of experimental diets

Ingredient and analysis	Starter (0 to 3 wk)	Grower (3 to 21 wk)	Breeder (21 to 61 wk)
		(%)	
Ground wheat	44.23	34.42	33.76
Ground corn	14.18	16.44	14.31
Ground oats	5.00	12.50	10.00
Soybean meal (48%C.P.)	17.34	7.37	13.42
Ground barley	5.00	10.00	15.00
Wheat shorts	7.50	15.00	1.29
Limestone	1.65	1.72	7.68
Dicalcium phosphate	1.58	0.86	1.06
Choline chloride premix[1]	0.50	0.50	0.50
Broiler microingredient premix[2]	0.50	0.50	
Layer microingredient premix[3]			0.50
Iodized salt	0.35	0.33	0.28
L-lysine HCL	0.03	0.16	0.03
DL methionine	0.14	0.13	0.17
Tallow	2.00	0.07	2.00
Rumensin	0.08	0.05	0.00
Calculated Analysis			
ME (Kcal/kg)	2875	2706	2750
CP (%)	15.49	14.98	15.49
Calcium (%)	1.00	0.90	3.19
Available P (%)	0.70	0.56	0.42
Lysine (%)	0.90	0.75	0.75
Methionine (%)	0.41	0.35	0.41

[1] Provided choline chloride in the diet at a level of 100 mg/kg.

[2] Broiler premix provided per kilogram of diet: vitamin A (retinyl acetate), 10,000 IU; cholcalciferol, 2,500 IU; vitamin E (DL-α-tocopheryl acetate), 35 IU; vitamin K, 2.0 mg; pantothenic acid, 14 mg; riboflavin, 5.0 mg; folacin, 0.8 mg; niacin, 65 mg; thiamine, 2.0 mg; pyridoxine, 4.0 mg; vitamin B12, 0.015 mg; biotin, 0.18 mg; iodine, 0.5 mg; Mn, 70 mg; Cu, 8.5 mg; Zn, 80 mg, Se, 0.1 mg; Fe, 100 mg.

[3] Layer premix provided per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU; cholcalciferol, 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 40 IU; vitamin K, 2.0 mg; pantothenic acid, 14 mg; riboflavin, 6.5 mg; folacin, 1.0 mg; niacin, 40 mg; thiamine, 3.3 mg; pyridoxine, 6.0 mg; vitamin B12, 0.02 mg; biotin, 0.2 mg; iodine, 0.5 mg; Mn, 75 mg; Cu, 15 mg; Zn, 80 mg, Se, 0.1 mg; Fe, 100 mg.

Table 3-2. Total feed allowances for birds on the fast feed (FF) or slow feed (SF) feeding regimen between 20 wk of age and sexual maturity (first oviposition)

	Experimental feeding regimen[1]	
Age (wk)	FF	SF
	(g per bird per d)	
20 to 21[2]	98	98
21 to 22	120	100
22 to 23	125	104
23 to 24	125	110
24 to 25	125	115
25 to 26	127	117
26 to 27	128	122
27 to 28	128	124

[1] FF = fast feed regimen; SF = slow feed regimen.

[2] Birds were acclimated to cages for 1 wk and experimental feeding regimens started at photostimulation (21 wk of age).

TABLE 3-3. Carcass traits and ovarian morphology of broiler breeder pullets at the onset of experimental feeding regimens (21 wk of age)

Parameter	
	(Mean ± SEM)
n	10
BW (g)	1939 ± 37
Breast muscle weight	
(g)	316.9 ± 7.2
(% of BW)	16.34 ± 0.22
Liver weight	
(g)	30.0 ± 0.6
(% of BW)	1.56 ± 0.04
Abdominal fatpad weight	
(g)	16.4 ± 2.9
(% of BW)	0.83 ± 0.14
Oviduct weight	
(g)	1.29 ± 0.34
(% of BW)	0.065 ± 0.016
Ovary weight	
(g)	0.91 ± 0.09
(% of BW)	0.046 ± 0.004

Table 3-4. Body weight, breast muscle, abdominal fatpad, and liver measurements of broiler breeders at sexual maturity following administration of an accelerated (fast feed) or gradual (slow feed) feeding regimen beginning at 21 wk of age

		Breast Muscle		Abdominal fatpad		Liver		
Source	Sexual maturity BW	Weight	Percentage[1]	Weight	Percentage	Weight	Percentage	Liver lipid content[2]
	(g)	(g)	(%)	(g)	(%)	(g)	(%)	(%)
Feed[3]								
FF	2733^a	423.3	16.17	48.7	1.85	47.4	1.81	6.66
SF	2618^b	422.5	16.38	47.5	1.83	47.9	1.86	6.53
SEM	39	7.6	0.21	2.8	0.09	1.0	0.04	0.70
SM Time[4]								
EARLY	2558^a	412.2^a	16.63^a	41.1^a	1.66^a	47.5	1.92^a	6.65
LATE	2787^b	434.0^b	15.97^b	54.5^b	2.00^b	47.9	1.76^b	6.69
SEM	29	6.7	0.20	2.5	0.09	1.0	0.04	0.71
Interaction								
FF-EARLY	2657^b	428.4	16.78	41.3	1.62	48.5	1.91	6.82
FF-LATE	2798^a	419.7	15.64	55.0	2.04	46.4	1.73	6.53
SF-EARLY	2460^b	396.1^b	16.47	41.0	1.70	46.4	1.94	6.21
SF-LATE	2776^a	448.4^a	16.28	54.0	1.95	49.4	1.79	6.85
SEM	42	9.5	0.28	3.46	0.12	1.4	0.06	1.00
	Probability							
Source of variation								
Feed	0.013	0.88	0.56	0.86	0.97	0.75	0.45	0.89
Time	0.0001	0.026	0.022	0.0004	0.010	0.79	0.007	0.86
Feed X SM time	0.043	0.003	0.095	0.92	0.49	0.080	0.76	0.64

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Percentage = tissue weight / BW X 100.

[2] Lipid percentage = liver lipid weight / liver weight X 100.

[3] Feeding regimens: FF = Fast feed; SF = Slow feed.

[4] Sexual maturity time: EARLY = first 50% of birds within each feeding regimen to reach SM; LATE = last 50% of birds within each feeding regimen to reach SM.

Table 3-5. Time of sexual maturity, oviduct, ovary, and stroma weights of broiler breeders at sexual maturity following administration of an accelerated (fast feed) or gradual (slow feed) feeding regimen beginning at 21 wk of age

Source	Days from PS to SM[1]	Oviduct Weight	Oviduct Percentage[2]	Ovary Weight	Ovary Percentage	Ovarian Stroma Initial[3]	Ovarian Stroma Bare[4]
	(d)	(g)	(%)	(g)	(%)	(g)	(g)
Feed[5]							
FF	20.9^{b}	64.5	2.46	59.7	2.28	6.26	2.19
SF	24.3^{a}	62.6	2.42	56.8	2.19	6.35	2.13
SEM	1.5	2.4	0.08	2.2	0.07	0.39	0.13
SM Time[6]							
EARLY	17.1^{b}	59.5^{b}	2.40	56.2	2.26	5.28^{b}	1.94^{b}
LATE	27.7^{a}	67.4^{a}	2.47	60.0	2.20	7.23^{a}	2.36^{a}
SEM	0.9	2.3	0.08	2.2	0.08	0.33	0.13
Interaction							
FF-EARLY	16.6^{b}	61.6	2.41	59.4	2.33	4.96	1.98
FF-LATE	21.5^{a}	66.9	2.50	59.9	2.23	7.36	2.38
SF-EARLY	17.6^{b}	57.5	2.39	53.1	2.20	5.59	1.90
SF-LATE	31.0^{a}	67.8	2.45	60.2	2.18	7.11	2.34
SEM	1.3	3.2	0.11	3.3	0.11	0.47	0.18
	Probability						
Source of variation							
Feed	0.006	0.62	0.74	0.34	0.39	0.70	0.77
Time	0.0001	0.019	0.51	0.24	0.58	0.0002	0.024
Feed X SM time	0.037	0.45	0.92	0.31	0.71	0.36	0.89

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Days from photostimulation to sexual maturity.

[2] Percentage = tissue weight / BW X 100.

[3] Initial stroma = ovary without the large yellow follicles (follicles >10mm diameter removed).

[4] Bare stroma = initial stroma without the small yellow follicles or large white follicles (follicles >2mm diameter removed).

[5] Feeding regimens: FF = Fast feed; SF = Slow feed.

[6] Sexual maturity time: EARLY = first 50% of birds within each feeding regimen to reach SM; LATE = last 50% of birds within each feeding regimen to reach SM.

Table 3-6. Follicle numbers, small follicle atresia, mean large yellow follicle weight, and hierarchy size of broiler breeders at sexual maturity following administration of an accelerated (fast feed) or gradual (slow feed) feeding regimen beginning at 21 wk of age

	Ovarian follicles[1]					LYF parameters		
Source	LYF number	SYF number	LWF number	MWF number	Small atretic follicles[2]	Mean weight	Hierarchy positions[3]	Number of hierarchies[4]
	(#)				(#)	(g)	(#)	(#)
Feed[5]								
FF	8.50	9.29	13.25	144.0	10.79	6.30	5.94	1.43
SF	8.17	10.54	13.58	144.9	12.92	6.16	5.89	1.38
SEM	0.30	0.94	1.19	12.8	2.19	0.14	0.18	0.07
SM Time[6]								
EARLY	8.09	7.83^a	11.14^b	101.8^b	9.26	6.27	5.84	1.39
LATE	8.55	11.82^b	15.47^a	183.5^a	14.18	6.20	5.99	1.43
SEM	0.31	0.86	1.12	9.7	2.18	0.13	0.18	0.06
Interaction								
FF-EARLY	8.27	6.91	10.45	96.3	7.27	6.55	5.64	1.47
FF-LATE	8.69	11.31	15.62	184.3	13.77	6.09	6.25	1.39
SF-EARLY	7.91	8.75	11.83	107.3	11.25	6.00	6.05	1.31
SF-LATE	8.42	12.33	15.33	182.6	14.58	6.30	5.73	1.47
SEM	0.44	1.21	1.58	13.7	3.07	0.19	0.26	0.09
	Probability							
Source of variation								
Feed	0.46	0.24	0.73	0.74	0.44	0.38	0.83	0.74
Time	0.29	0.002	0.009	0.0001	0.12	0.67	0.57	0.84
Feed X SM time	0.92	0.74	0.60	0.65	0.61	0.053	0.076	0.046

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] LYF = large yellow follicles (>10mm diameter); SYF = small yellow follicles (5-10mm diameter); LWF = large white follicles (3-5mm diameter); MWF = medium white follicles (1-3mm diameter).

[2] Number of atretic follicles < 5 mm in diameter.

[3] Number of follicle groups within 1 g.

[4] Hierarchies calculated as LYF divided by positions.

[5] Feeding regimens: FF = Fast feed; SF = Slow feed.

[6] Sexual maturity time: EARLY = first 50% of birds within each feeding regimen to reach SM; LATE = last 50% of birds within each feeding regimen to reach SM.

Table 3-7. Distribution of ingested radiolabel between the liver, ovary, and abdominal fatpad at processing in broiler breeders at sexual maturity following administration of an accelerated (fast feed) or gradual (slow feed) feeding regimen beginning at 21 wk of age

	Deposition of radioactivity[1]		
Source	Liver	Ovary	Abdominal fatpad
	(%)		
Feed[2]			
FF	18.2	34.9	46.9
SF	17.8	36.0	46.2
SEM	1.3	1.2	1.5
SM Time[3]			
EARLY	19.5	39.4[a]	41.1[b]
LATE	16.6	31.4[b]	52.0[a]
SEM	1.3	1.2	1.5
Interaction			
FF-EARLY	18.6	38.1	43.3
FF-LATE	17.9	31.6	50.5
SF-EARLY	21.3	40.7	38.9
SF-LATE	15.3	31.2	53.5
SEM	1.8	1.8	2.0
	Probability		
Source of variation			
Feed	0.82	0.53	0.74
Time	0.54	0.0001	0.0001
Feed X SM time	0.25	0.41	0.089

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Deposition calculated as a percentage of total measured ^{14}C activity in the liver, the ovary (including ovulated follicles), and the abdominal fatpad.

[2] Feeding regimens: FF = Fast feed; SF = Slow feed.

[3] Sexual maturity time: EARLY = first 50% of birds within each feeding regimen to reach SM; LATE = last 50% of birds within each feeding regimen to reach SM.

Table 3-8. Plasma hormone concentrations and plasma lipid and VLDL parameters of broiler breeders at sexual maturity following administration of an accelerated (fast feed) or gradual (slow feed) feeding regimen beginning at 21 wk of age

	Plasma	Plasma	Plasma	Plasma	VLDL traits at PS			VLDL traits at SM		
Source	Estradiol-17β at PS	Estradiol-17β at SM	lipids at PS	lipids at SM	Lipid content	TG content[1]	TG:PL ratio	Lipid content	TG content[1]	TG:PL ratio
	(pg/mL)		(mg/mL)		(mg/mL)			(mg/mL)		
Feed[1]										
FF	52.1	81.7	6.91	22.9	1.22	0.48	0.87	14.31	9.18	2.14
SF	55.4	100.8	7.57	23.3	1.47	0.58	0.88	15.31	9.99	2.37
SEM	4.5	8.8	0.28	1.7	0.13	0.08	0.13	1.35	0.97	0.14
SM Time[2]										
EARLY	59.4	86.2	7.50	21.22	1.52^a	0.65^a	1.00	12.95^b	8.35	2.22
LATE	48.1	96.3	6.97	24.94	1.16^b	0.41^b	0.75	16.68^a	10.82	2.29
SEM	4.4	8.8	0.28	1.66	0.13	0.08	0.13	1.35	1.00	0.14
Interaction										
FF-EARLY	51.4	72.9	7.00	20.79	1.36	0.53	0.90	12.14	7.76	2.07
FF-LATE	52.8	90.6	6.81	24.90	1.08	0.42	0.83	16.49	10.60	2.21
SF-EARLY	67.4^a	99.5	8.01	21.66	1.68	0.77	1.10	13.75	8.94	2.37
SF-LATE	43.3^b	102.0	7.13	24.98	1.25	0.39	0.67	16.87	11.04	2.37
SEM	6.3	12.4	0.40	2.34	0.18	0.12	0.19	1.91	1.39	0.20
	Probability									
Source of variation										
Feed	0.61	0.13	0.11	0.84	0.20	0.41	0.93	0.53	0.55	0.27
Time	0.081	0.42	0.19	0.12	0.043	0.0476	0.19	0.046	0.075	0.74
Feed X SM time	0.050	0.54	0.39	0.87	0.75	0.27	0.35	0.69	0.78	0.75

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Feeding regimens: FF = Fast feed; SF = Slow feed.

[2] Sexual maturity time: EARLY = first 50% of birds within each feeding regimen to reach SM; LATE = last 50% of birds within each feeding regimen to reach SM.

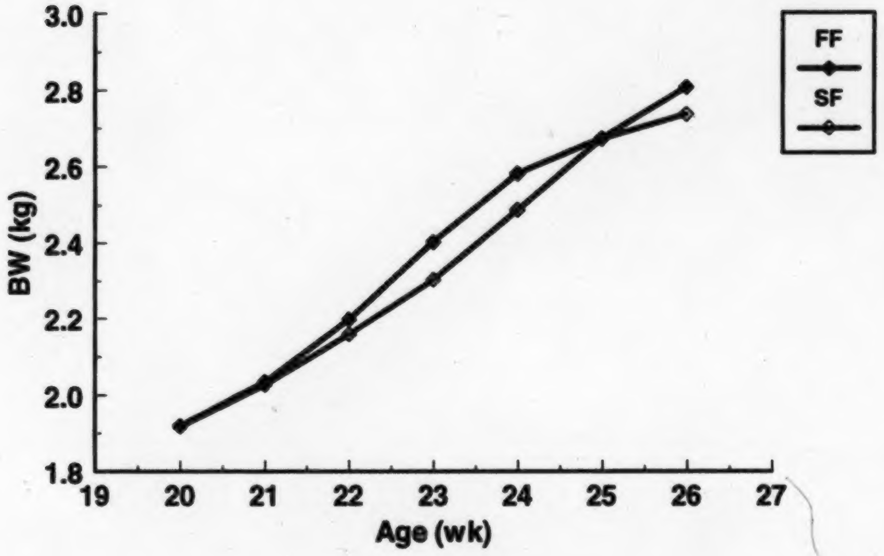

Figure 3-1. Body weight curve of broiler breeders on an accelerated (fast feed) or gradual (slow feed) feeding regimen between 21 wk of age and sexual maturity.

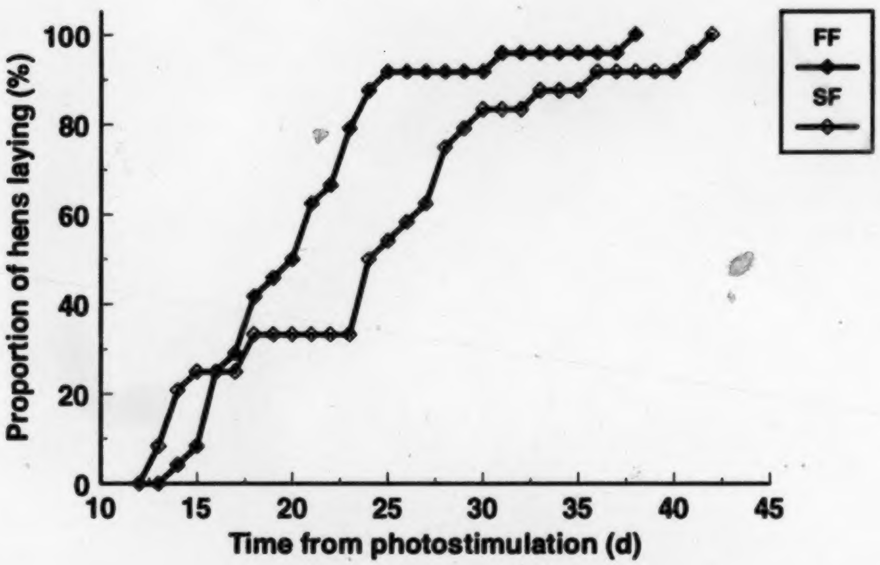

Figure 3-2. Additive curve of proportion of broiler breeders reaching sexual maturity following administration of an accelerated (fast feed) or gradual (slow feed) feeding regimen beginning at 21 wk of age.

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4. EFFECTS OF FEEDING PROGRAM AND ESTRADIOL-17β IMPLANTS ON CARCASS AND REPRODUCTIVE TRAITS IN 40 WEEK OLD BROILER BREEDERS

4.1 INTRODUCTION

Broiler breeder pullets are routinely feed restricted from an early age to reduce reproductive problems relating to selection for growth. Overfeeding birds as they are reaching sexual maturity results in the production of excess LYF on the ovary, which will be more likely to be arranged in multiple hierarchies of large follicles (Hocking *et al.*, 1989; Yu *et al.*, 1992). This paired follicle production is related to reduced production of settable eggs. The adverse effects of *ad libitum* feeding of birds occur primarily before peak egg production (Yu *et al.*, 1992). By 44 wk of age, overfeeding hens will increase LYF production within 14 d, but to a lesser degree than at sexual maturity (Robinson *et al.*, 1993). In 54 wk old breeders, McGovern *et al.* (1997) found that increasing feed allowance did not alter ovarian morphology.

Treatment of hens with exogenous estradiol-17β at the end of the breeder period (59 wk of age) causes increased LYF weights, but otherwise has no effect on ovarian morphology (Renema *et al.*, 1997). Whereas the number of LYF at sexual maturity is correlated with BW in birds that come into production earlier within a flock, the LYF number of the later maturing birds correlated best with plasma estradiol-17β concentration (Renema, Thesis: Chapter 3). The effects of exogenous estrogen on ovarian morphology in birds after peak production, when the ovary is still responsive to changes in energy balance, is not known. This experiment examined the effects of a 2 wk treatment with exogenous estradiol-17β on carcass measurements, ovarian morphology, and plasma lipid parameters at 42 wk of age in combination with the effects of a standard or enriched diet fed from 21 wk of age. The dietary treatment was intended to test the reproductive sensitivity of a high breast yield broiler breeder strain to moderate overfeeding conditions and the estradiol-17β treatment was intended to stimulate ovarian yolk deposition and alter lipid utilization within standard and moderately overfed birds.

4.2 MATERIALS AND METHODS

Stocks and Management

A total of 32 Shaver[1] experimental high breast yield broiler-breeder pullets of similar BW were housed in individual laying cages at 20 wk of age. Birds were randomly assigned to either a standard breeder diet (STD) of 2750 kcal/kg and 15.7% C.P., or a to a concentrated diet (CON) of 2900 kcal/kg and 16.5% C.P. (Table 4-1). Diet treatments began at the time of photostimulation (21 wk of age). The STD birds were used to determine feed allocation following industry BW guidelines. All birds were fed individually. The CON birds received the same daily feed allocation as the STD birds. Individual BW were recorded on a weekly basis. Hens were artificially inseminated with 0.05 mL pooled undiluted semen weekly. At 40 wk of age, one half of the hens in each feeding group were randomly assigned to either a blank (BL) or an estradiol-17β (E2) subcutaneous silastic implant group. Eggs collected during the 2 wk implantation period were set and hatched at a commercial hatchery[2]. At transfer, marked eggs were placed in 13 cm diameter wire mesh hatching rings to allow for chick identification following hatch. The experimental protocol was approved by the Animal Policy and Welfare Committee of the Faculty of Agriculture, Forestry and Home Economics of the University of Alberta.

Egg Production Parameters

Birds were weighed at sexual maturity, which was defined as the age at first oviposition. Individual egg weights were recorded for each bird until it laid two sequential eggs greater than the 52 g minimum shipping weight, and a single egg weight per bird recorded on a weekly basis thereafter. Bird age at the time of the second 52 g egg was recorded. Individual daily egg production records indicating egg type (normal or unsettable) were maintained. Laying records were subjected to sequence analysis. A sequence was defined as a period of consecutive ovipositions separated by a pause of one or more days. Mean sequence length was calculated as a mean of all sequences occurring for each bird. Mean weekly sequence length was determined by assigning each day a sequence length number based on the total length of the sequence encompassing that day and analyzing the mean of these values for each bird on a weekly basis. Mean pause length and weekly pause length were determined in a similar fashion. The prime sequence was defined as the longest egg sequence which occurred near the time of peak egg production. The hatchability of eggs set for each bird was calculated as the number of viable chicks, excluding cull chicks, obtained from the total number of eggs set.

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[1] Shaver Poultry Breeding Farms Ltd., Box 400, Cambridge, ON, N1R 5V9
[2] Lillydale Co-operative Ltd., Edmonton, AB, T5C 1R9

Hormone Treatment

Solid silastic pellets containing estradiol-17β or no steroid (blank) were manufactured as described by Renema *et al.* (1997). Hormone implant size was based on previous unpublished work which revealed that the implants increased plasma estradiol-17β concentration by 120 pg/mL. Birds were weighed and an initial 10 cc blood sample taken for plasma lipid and hormone analysis prior to estradiol-17β or blank Silastic pellet implantation at 0800 h. Birds received either the 2 X 2 X 18 mm hormone pellet (contained 7.2 mg estradiol-17β/pellet) (E2 birds) or a similarly sized blank pellet (BL birds). The implant was placed subcutaneously through a 2 mm incision caudal to the cervical feather tract as previously described (Renema *et al.*, 1997).

Birds were maintained on their feeding regimens with the implants for 14 d. Blood samples were taken at 7d for hormone quantification and at 14 d for analysis of hormone and plasma traits as above. At 0800 h birds were euthanatized by cervical dislocation, and individual BW was recorded. The breast muscle, liver, abdominal fatpad, oviduct, and ovary were removed and weighed. The liver was stored at -15°C until lipid content was determined. The number and weight of large yellow ovarian follicles (LYF) (> 10 mm diameter), and the number of small yellow follicles (SYF) (5-10 mm diameter), and large white follicles (LWF) (2-5 mm diameter) were recorded. Follicle size classifications were based on previous reports (Robinson and Etches, 1986), although in the current study the range of diameters used was continuous. Stroma weight was recorded with the LYF removed (initial stroma) and again when the SYF and LWF had also been removed (bare stroma) to assess treatment effects of large and small follicle types separately. An assessment of the potential for multiple ovulations to occur was made by assigning LYF of similar size (differing by less than 1 g or 1 mm diameter) to the same position in the hierarchy as reported previously (Renema *et al.*, 1995). Total number of positions and proportion of follicles in a multiple hierarchical arrangement were recorded. Number of complete hierarchies of LYF was calculated by dividing LYF number by the number of positions in the hierarchy. The incidence of internal ovulation (as evidenced by yolk residue in the body cavity) and the incidence of follicular atresia of the yellow follicles (> 5 mm diameter) was assessed. Small follicles (< 5 mm diameter) with a discolored and/or shrunken appearance were considered atretic (Gilbert *et al.*, 1983).The extent of small follicle atresia was assessed by counting all visible atretic follicles.

Liver lipids were quantified using standard chemical analysis procedures (AOAC, 1980) using 2 g of sample in duplicates. True tissue lipid content was calculated by adjusting recorded values to account for moisture loss during the tissue drying process.

Blood Plasma Analysis

Blood samples were taken from the brachial vein using EDTA-coated vacuum blood collection tubes with 21 gauge needles. Blood was centrifuged at 1500 *g* for 20 min at 3°C. A preservative solution of streptomycin sulfate and penicillin-G (50 U each/mL stock) was added to plasma at a level of 20 µL/mL to limit bacterial and mold growth. Plasma for subsequent lipid quantification was stored at -30°C. Plasma undergoing VLDL lipid quantification and analysis was further treated with 1 µL/mL of a 1M benzamidine solution and 5 µL/mL of a 0.2M phenylmethylsulfonyflouride (PMSF) solution to limit protease activity. Two mL of plasma were overlayered with 2 mL of 0.85% KBr solution (density = 1.0063) and ultracentrifuged as described by Renema *et al.* (1997).

Total plasma lipids and very low density lipoprotein (VLDL) lipid weight was determined by Folch lipid extraction. Chloroform was evaporated from the 15 mL extracted aliquot under a nitrogen stream on a hot-plate (70°C at surface) until a constant weight was achieved. The VLDL lipid class contents were separated by thin layer chromatography on silica gel chromatorods using a benzene:chloroform:acetic acid: methanol (88.9:10.5:3.9:2.6 vol/vol) developing solvent system. Lipids in each class were quantified by IATROSCAN (TH-10 TLC/FID analyzer)[3] as described by Renema *et al.* (1997).

Plasma estradiol-17β concentration was determined using a RIA[4] kit as described by Renema *et al.* (1997). All samples were analyzed in a single RIA with an intra-assay coefficient of variation of 1.88%. Plasma luteinizing hormone (LH) concentration was determined in a single RIA using the method of Krishnan *et al.* (1994) for which the intra-assay coefficient of variation was 10.67%.

Statistical Analysis

The experiment was a 2X2 factorial design with the main effects, Feed and Hormone. Treatments were applied to the upper and lower levels of a battery of cages in a completely randomized design. Data on the feeding regimens prior to 40 wk of age were evaluated by one-way analyses of variance using the ANOVA procedures of SAS® (SAS Institute, 1994). Source of variation for the parameters compared at 40 wk of age was feeding regimen. Sequence and pause length analyses were performed by comparing individual means generated for the two 8 wk time periods between 24 and 40 wk of age and for the entire 16 wk period.

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[3] Iatron Laboratories, Inc., Tokyo, 101 Japan
[4] Kit Number TKE25, Diagnostic Products Corp., Los Angeles, CA 90045-5597

Data were evaluated at processing by two-way analysis of variance using the General Linear Models (GLM) procedures of SAS® (SAS Institute, 1994). Sources of variation at processing were feeding regimen, hormone, and the interaction feeding regimen X hormone. Differences between means were evaluated with Fisher's protected LSD procedure (Peterson, 1985). The error variation for all variables consisted of the variation between birds within the interaction.

Pearson correlation coefficients (Steel and Torrie, 1980) were computed between reproductive, carcass, and plasma parameters across all treatments and within each feeding regimen. Following data analysis within the main effects, birds of all treatments were pooled and ranked into the upper and lower 25% and 50% for the triglyceride:phospholipid (TG:PL) ratio of the plasma VLDL lipids, to better define the effects of flock variability on the relationship between plasma lipid characteristic and carcass and reproductive parameters during a feed or sex hormone challenge. Birds of the upper 25% and 50% of the ranking were compared to the lower 25% and 50% for each parameter by subjecting them to one-way Analysis of Variance using SAS® (SAS Institute, 1994). Two birds were removed from the experiment due to normal mortality. As a result, SEM values presented were based on the treatment group within the main effect with the fewest birds. Unless otherwise stated, all statements of significance were based on testing at the $P < 0.05$ level.

4.3 RESULTS AND DISCUSSION

Initial Reproductive and Plasma Traits

The CON birds reached sexual maturity 2.3 d prior to the STD birds at a 2.3% greater BW (Table 4-2). These values were not significantly different and were indicative of the similarities between the birds on these two feeding regimens. The birds needed an average of 30 d after photostimulation to reach sexual maturity, which is similar to values reported for flocks from the same facility (Robinson *et al.*, 1995, 1996), and 1 wk longer than values reported for a companion flock reared concurrently (Renema, Thesis: Chapter 3). Fattori *et al.* (1991) increased dietary energy content by 8% and reported birds to come into lay 1 wk sooner than control birds. The BW of the STD and CON birds were up to 10% higher than those reported by Robinson *et al.* (1996) for birds photostimulated at a similar age. Both diets of the current experiment contained 4% tallow compared to the 1% (Zuidhof *et al.*, 1995) to 2% (Renema *et al.*, 1997) more typically used in order to keep experimental diet fat contents similar. The readily available energy of the increased dietary fat content may have contributed to the greater BW gains by sexual maturity observed in the current study. Between photostimulation and first oviposition, STD birds consumed 527 g C.P. and 9234 Kcal M.E. whereas CON birds consumed 507 g C.P. and 8904 Kcal M.E. Nutrient intakes were not different, indicating that STD and CON birds required the same amount of nutrients for sexual maturation.

Body weight profiles for the breeder period are presented in Figure 4-1. The extra nutrient density of the CON feeding regimen compared to the STD regimen caused their growth curve to appear to separate above the STD bird curve. The maximum separation was 4.0% at 25 wk of age (P=0.07). The BW curves remained similar through to 40 wk of age. The CON birds were consuming 5.3% more protein and energy than STD birds. The additional nutrients were presumably divided between the increased metabolic needs of these numerically heavier birds and processes such as reproduction. A BW influence on reproduction may be suggested by the 6 d separation in time to consecutive 52 g eggs (46.1 *vs.* 40.2 d for STD and CON birds, respectively) (P=0.11) (Table 4-2).

Egg production to 40 wk of age was similar in STD and CON birds (Table 4-2). The flock production curve (Figure 4-2) shows that the CON birds may have initially laid at a higher rate, whereas after 32 wk of age the STD birds appear to be laying better. Experiments with *ad libitum* birds show similar results, with the overfed birds coming into lay more quickly than restricted birds, but laying more poorly later in the breeder period (Robinson *et al.*, 1991; Yu *et al.*, 1992), due in part to increased inter-sequence pause length (Robinson *et al.*, 1991). The production of birds fed 8% above standard by Fattori *et al.* (1991) also followed this pattern.

Despite only minor differences in their nutrient intake, the egg laying patterns of the CON birds of the current study were significantly different than those of the STD birds (Table 4-2). These effects occurred late in the production period (32 to 40 wk of age). At this time mean weekly sequence length in STD birds was greater (4.84 *vs.* 4.09 d for STD and CON birds, respectively) and inter-sequence pause length shorter (1.45 *vs.* 1.72 d for STD and CON birds, respectively) than those of the CON birds. The superior sequence lengths of STD birds can be seen in the latter half of the weekly mean sequence profile (Figure 4-3). Compared to the overall individual sequence length means used to calculate mean sequence length, the mean weekly sequence length is calculated to represent the laying patterns of the flock at weekly intervals (Table 4-2). This results in higher values, as longer sequences are counted in subsequent weeks, and an accurate reflection of sequence or pause length in time. Although *ad libitum* fed birds have been reported to lay fewer settable eggs later in lay, the negative effects of overfeeding are primarily expressed in early lay, prior to peak production (Yu *et al.*, 1992). It has been speculated that the reason *ad libitum* feeding may have less effect as the bird ages is due to a decreasing sensitivity of the ovary to increasing feed intake (Hocking, 1996). The egg laying patterns of the birds in the current study did not differ in early lay, as indicated by their similar prime sequence lengths (mean of 11.3 d) and early weekly mean sequence length (mean of 7.22 d) (Table 4-2). Unsettable egg production, a trait normally associated with overfeeding broiler breeders, did not differ significantly between feeding regimens (5.3 *vs.* 8.6 in STD and CON birds, respectively) (P=0.18).

Plasma estradiol-17β concentration at time of hormone implantation (40 wk of age) was 110 and 107 pg/mL in STD and CON birds, respectively (Table 4-3). Plasma lipid concentration was also similar in STD and CON birds, whose plasma contained 29.1 and 27.1 mg/mL of lipid, respectively. Plasma LH concentration was numerically increased in CON birds by 19%

(P=0.22). In work with dwarf broiler breeders, Dunn *et al.* (1990) reported that birds receiving supplemental energy in the form of maize oil had higher plasma LH concentrations following photostimulation. It was speculated that more intense feed restriction may be activating pathways inhibitory to plasma LH.

Carcass and Reproductive Morphology at Processing

Body weight at processing was 4.6% greater in CON birds than in STD birds (Table 4-4). Prior to this the BW range between CON and STD birds increased from 2.2% at 40 wk of age to 3.1% at 41 wk of age (P=0.12). The liver weights of CON birds were heavier than those of STD birds at 42 wk of age (Table 4-4). Relative liver lipid content was 16.5% compared to 11.5% in CON *vs.* STD birds, respectively. The difference due to hormone treatment did not significantly differ (P=0.14), with the E2 bird livers containing 15.2% lipid compared to 12.8% in the BL bird livers. Robinson *et al.* (1993) reported that after 7 d of *ad libitum* feeding, 44 wk old birds were depositing excess energy into the liver, and by 14 d the abdominal fatpad was also expanding. In the current experiment, birds did not receive supplemental nutrients following implantation, so any changes in tissue measurements are due to altered growth patterns and lipid allocation. Estradiol-17β treatment in 59 wk old broiler breeders continuing on the same feeding regimen resulted in an increase in liver uptake of radiolabeled lipid from 11% to 20%, which was primarily at the expense of abdominal fatpad deposition (Renema *et al.*, 1997). Differences in liver weight and lipid content in the current experiment may have existed prior to hormone treatment, but were possibly accentuated by estradiol-17β treatment. Liver lipid weight as a percentage of liver weight was correlated with ovary weight (r=0.513; P=0.004) and ovarian stroma weight (r=0.382; P=0.04). This important link between the site of yolk lipid synthesis and deposition was independent of BW.

There were no significant differences in absolute or relative oviduct, ovary, or ovarian stroma weights (Table 4-5). If the 2 wk of E2 treatment was causing negative feedback at the level of the hypothalamus, the effects of reduced LH and follicle stimulating hormone (FSH) secretion should be apparent in the size of the bare stroma, which represents the thousands of developing follicles less than 2 mm in diameter. Bare stroma weight was 5.25 g in BL birds and 5.41 g in E2 birds, indicating that if negative feedback effects were present in the ovary, they were not great enough to be detected at the macroscopic level of tissue weight.

In an estradiol-17β implant study in 59 wk old broiler breeders, Renema *et al.* (1997) found that hormone treatment increased yolk deposition, as indicated by a 9.7% increase in mean LYF weight. Robinson *et al.* (1993) observed an increase in yolk deposition due to 7 d of overfeeding and increase in LYF number of 1.2 by 14 d. In the current experiment, neither LYF number nor mean weight was significantly affected by feeding regimen or hormone treatment (Table 4-6). The arrangement of LYF was affected by feeding regimen, however, as the CON birds had a greater number of hierarchies of LYF present on the ovary than the STD birds. Whereas LYF numbers were similar, with 5.94 and 5.97 LYF on STD and CON ovaries, respectively, the CON ovaries had 0.77 more paired follicles present, which increased the hierarchy number to 1.1 from 1.0 in the STD birds. Increased pairing of follicles disrupts normal ovarian function through increased production of defective eggs (Hocking *et al.*, 1987, 1989; Yu *et al.*, 1992). Within 7 d of overfeeding, Robinson *et al.* (1993) reported the incidence of simultaneous development in 44 wk old birds to be increased from 3% of control birds to 50% in *ad libitum* fed birds without affecting LYF number. They stated that the number of LYF may be less important than the uniformity of spacing of the follicles in ensuring the regular, single ovulation of follicles.

The number of LYF at this age (42 wk) (Table 4-6) is in agreement with values reported for similarly aged birds (Hocking *et al.*, 1987; Robinson *et al.*, 1993), and values reported for 59 wk old birds by Renema *et al.* (1997). There were fewer LYF than have been reported at either sexual maturity or at 33 wk of age (Yu *et al.*, 1992). The number of white follicles between 2 and 5 mm in diameter was double the number reported by Hocking *et al.* (1987) in a different strain. The number of atretic follicles under 5 mm diameter was triple that reported for 45 wk old birds (Hocking *et al.*, 1987) and quadruple levels observed at sexual maturity (Renema, Thesis:

Chapter 3). It is clear that increased rates of follicular atresia may be related to age-related declines in egg production, as suggested by Palmer and Bahr (1992).

Production Parameters Following Hormone Implantation

The STD birds produced 4.62 eggs in the first week of hormone treatment compared to 4.86 eggs by CON birds (P=0.58). In the second week, egg numbers were 4.70 for STD hens and 3.88 for CON hens (P=0.060). This apparent decline in egg production was linked to estradiol-17β treatment (P=0.18), with the largest production loss in the CON-E2 birds, who produced 5.0 eggs in wk 1 compared to 3.8 in wk 2. Hatchability during the implantation period averaged 86.3% in the two feeding regimens, with no significant effect of E2 treatment (Table 4-7).

Egg weights during the implantation period were compared with those of the 2 wk period prior to it (38 to 40 wk of age) (Table 4-7). Whereas no previous differences existed between egg weights of birds assigned to the BL and E2 treatments, hormone treatment resulted in a significantly reduced egg weight in E2 compared to BL bird eggs during the implant period (66.1 *vs.* 67.4 g, respectively).

Feeding regimen continued to have a significant effect on egg weight between 40 and 42 wk of age, with mean CON treatment egg weight being 67.7 g compared to 65.8 for STD treatment eggs (table 4-7). This difference carried forward to egg weight at transfer from the incubator to the hatcher, chick weight, and chick weight relative to egg weight. E2 treatment caused the smaller eggs of the E2 treatment to behave differently during incubation, however. The smaller E2 treatment eggs weighed the same as BL treatment eggs at transfer (mean = 59.6 g) and produced chicks of the same weight as BL treatment eggs (mean = 48.1 g), resulting in chick weight relative to egg weight being greater in eggs of E2 birds compared to BL birds (72.4% *vs.* 71.9% for E2 and BL, respectively). This difference was driven primarily by the significant interaction within CON hormone treatments. The chick weight proportion of CON-E2 treatment eggs was 74.1% compared to 71.4% for CON-BL treatment eggs. As indicated by the weight of the F1 follicle (largest ovarian LYF), the yolk size of BL and E2 birds eggs was similar (19.2 g *vs.* 19.1 g for BL and E2 F1 follicles, respectively). E2 treatment may have modulated lipid allocation and utilization in CON bird ovaries in a similar way to its apparent affects on liver lipids. The implications of this effect of increased estradiol-17β concentration are the possibility of an enhanced chick weight without an increased yolk or egg weight.

Plasma Hormone and Lipid Parameters at Processing

The difference in plasma estradiol-17β concentration between implantation and processing was 54.4 pg/mL greater in E2 birds than in BL birds. This maintained estradiol-17β concentrations at physiological levels, but well below the target concentration increase of 120 pg/mL (Table 4-8). This implant was more effective for Renema *et al.* (1997), who found it to increase plasma estradiol-17β concentrations by 115 pg/mL in 59 wk old broiler breeders. Neither feeding regimen nor hormone treatment had any significant effects on plasma lipid concentration, plasma VLDL traits at processing (Table 4-8) or on differences between initial (40 wk) and processing (42 wk) values. Plasma hormone concentrations at day 7 and 14 of the implant period were not different (data not shown), indicating that the estradiol-17β supplied by the implant remained constant throughout the experimental period and that it exerted a constant effect on endogenous LH levels.

Plasma LH concentration was numerically reduced by 7.1% and 9.7% during the hormone implant period in STD and CON birds, respectively. This apparent decrease may be due to negative feedback effects of long term elevated estradiol-17β concentrations on gonadotrophin production and release by the pituitary. Apart from the CON-E2 birds, whose plasma lipids increased by 9.0 mg/mL during the implant period, plasma lipid concentrations remained stable. The TG and PL concentrations and TG:PL ratio of plasma VLDL had the same means at processing as at time of hormone implantation (Table 4-8).

Plasma estradiol-17β levels were loosely correlated with plasma lipid concentration at processing (r=0.314; P=0.09). A stronger relationship existed between plasma LH at processing and plasma lipid concentration both at processing (r=0.423; P=0.02), and at time of implantation (r=0.631; P=0.0002). Initial estradiol-17β and LH concentrations were not correlated with plasma lipids (P=0.31 and P=0.28, respectively). It is not clear how the E2 treatment influenced LH levels to change in a way that related to initial plasma lipid concentrations, although it may be indicative of an interaction between nutritional and reproductive pathways.

Several parameters measured at processing correlated with time of sexual maturity (mean = 30 d after photostimulation). Final estradiol-17β concentration was negatively correlated with day of first oviposition (r=-0.379; P=0.04), whereas the initial 40 wk values were not (P=0.98). Whereas the initial VLDL-TG:PL ratio was not related to day of first egg (P=0.89), the final TG:PL ratio approached significance in its correlation with sexual maturation date (r=-0.349; P=0.059). It appears that birds which achieved sexual maturity early were more likely to have elevated plasma estradiol-17β concentrations and a potentially higher VLDL-TG:PL ratio. Ultimately the association between traits measured at 42 wk of age and sexual maturity may be due to BW and carcass composition effects near photostimulation. Birds reaching sexual maturity earlier are generally heavier and contain larger lipid stores than their non-laying counterparts (Brody *et al.*, 1984). Methods these birds use to respond to a feed or hormonal challenge later in life may already be determined by this age.

The VLDL-TG:PL ratio at processing was found to be correlated more to ovarian and carcass morphology traits than any other parameter examined. To more clearly examine its effects, birds of all treatments were pooled and ranked by their final VLDL-TG:PL ratio and the upper 50% (U-50) and upper 25% (U-25) of the birds were compared to the lower 50% (L-50) and lower 25% (L-25) of the birds (Table 4-9). The TG:PL ratio was not affected by plasma estradiol-17β concentration (P=0.82), BW (P=0.47), or plasma VLDL concentration (P=0.20), whereas day of first egg was numerically affected (28.0 d *vs.* 31.7 d for U-50 and L-50, respectively) (P=0.09). The mean VLDL-TG:PL ratio in the U-50 and L-50 groups were 2.95 and

1.94, respectively. The change in the TG:PL ratio between 40 and 42 wk of age accounted for this difference, with the ratio of the U-50 birds increasing by 0.64 while the L-50 ratio dropped by 0.47 (Table 4-9). The primary effects of a high VLDL-TG:PL ratio (U-50) were an increased relative abdominal fatpad weight (5.33% *vs.* 4.68% for U-50 and L-50, respectively) and numerically increased absolute fatpad weight (P=0.06), and a reduced absolute ovary weight (66.1 g *vs.* 75.5 g for U-50 and L-50, respectively) and ovarian stroma weight (6.6 g *vs.* 7.7 g for U-50 and L-50, respectively). With the reduced ovary size, there was a concomitant reduced mean LYF weight in U-50 birds in combination with a smaller mean LWF weight.

Although overall follicle numbers were not affected by VLDL-TG:PL ratio, numbers of large atretic follicles (>5mm diameter) were numerically reduced in U-50 birds (P=0.13) and small atretic follicle numbers were significantly reduced (39.0 *vs.* 50.7 for U-50 and L-50, respectively) (Table 4-9). The U-25 and L-25 groups have an even greater separation between values of most traits, but fewer significant differences due to high SEM values. Large atretic follicle number (P=0.06) and small atretic follicle number (P=0.025) were the only parameters for which the degree of significance increased in the U-25 and L-25 comparison. Timing of sexual maturity has previously been reported to affect the rates of small follicle atresia, with the later birds having increased atresia in small follicles (Renema, Thesis: Chapter 3). Increased incidence of small atretic follicles is associated with lower LYF numbers (Hocking *et al.*, 1987). In the current study, day of first egg was numerically greater in the L-50 than in the U-50 birds by 3.7 d. This apparent delay may relate to the greater levels of small follicle atresia in the L-50 birds.

In a study of lipid allocation in older birds, Renema *et al.* (1997) reported that deposition of lipid in the ovary occurred at the expense of both abdominal fatpad and liver deposition. The inverse relationship between ovary weight and abdominal fatpad weight in the current study fits that model. Liver-synthesized VLDL for ovarian deposition has a smaller diameter than other VLDL (Griffin *et al.*, 1982) and is preferentially taken up by ovarian follicles (Schneider *et al.*, 1990). In the L-50 birds, both ovary mass and mean LYF weight was greater than in U-50 birds, whereas the VLDL-TG:PL ratio was lower (Table 4-9). The VLDL-TG:PL ratio would be expected to be lower in these birds as a result of the production of a greater proportion of smaller VLDL particles containing less TG (Chapman, 1980; Bacon *et al.*, 1982). A low VLDL-TG:PL ratio has also been reported to be correlated with the highest egg production rates in feed restricted birds (Renema *et al.*, 1997). Although egg production did not differ between U-50 and L-50 groups, the inverse correlation of both mean sequence length (P=0.07) and prime sequence length (P=0.06) with VLDL-TG:PL ratio approached significance. Plasma estradiol-17β concentration at processing was the plasma trait most correlated with egg production (r=0.383; P=0.037). Higher final estradiol-17β concentrations were positively related to superior egg production in this flock.

The estradiol-17β implants did not produce the same effect in all birds. Individual variability existed in the response to the exogenous hormone challenge which ultimately caused both plasma hormone concentrations and VLDL-TG:PL ratio to change in a way that related to ovarian morphology. The state of the ovary at sexual maturity has been reported to relate to the future reproductive performance of the birds, with differences of as little as one extra LYF resulting in a 10 egg reduction in subsequent egg production (Robinson *et al.*, 1995). The maturation of the physiological pathways controlling ovarian morphology may permanently affect the function of particular aspects of lipid synthesis or hormone response. Renema *et al.* (1997) reported that the best egg layers in the flock underwent the greatest increases in plasma lipid concentrations due to a feed or estradiol-17β challenge. Birds in the current study may have modulated the effects of the hormone implants on plasma hormone concentrations through differential steroid clearance rates. This may occur through processes such as rate of intestinal passage, which can affect steroid recycling (Adams *et al.*, 1994). The current experiment was designed specifically to avoid treatment-based variation in intestinal rate of passage due to feed volume, however, by utilizing qualitative rather than quantitative differences in diets. Alternatively, control of ovarian morphology may be influenced by developmental or nutritional effects on steroid-carrying capacity of the blood through hormonally-induced changes to sex hormone binding globulins (SHBG), or by the extent of the influence of stored lipids in decreasing circulating hormones. The ability of the estradiol-17β implants to raise plasma hormone concentrations was numerically more effective in STD than in CON birds. The enriched nutrient environment of the CON bird blood may be affecting steroid carrying capacity. The concentration of SHBG has been reported to inversely relate to plasma insulin concentration in a BW and carcass fat content-dependent manner (Botwood *et al.*, 1995).

The use of a standard feed restriction diet or a nutritionally-enriched concentrated diet did not significantly affect settable egg production in this experimental high breast yield broiler breeder strain. Laying patterns of CON birds demonstrated some of the negative effects associated with overfeeding, however, such as reduced sequence length, and increased pause length. Egg and chick size was greater in CON birds whereas fertility was not negatively affected. The ovarian morphology of this strain appears relatively insensitive to excess nutrient availability and to estradiol-17β augmentation. The VLDL-TG:PL ratio at processing was a good indicator of ovary size and reproductive fitness. The timing and sequence of events during sexual maturation appear to have effects that go far beyond peak production, and may affect how a bird will respond to a feed or hormone challenge later in lay.

Table 4-1. Composition and analysis of experimental breeder diets

Ingredient and analysis	Standard	Concentrated
	(%)	
Ground wheat	29.60	52.91
Ground corn	10.00	10.00
Ground oats	10.00	3.26
Soybean meal (48%C.P.)	13.84	15.61
Ground barley	15.00	3.00
Wheat shorts	7.36	1.00
Limestone	7.72	7.69
Dicalcium phosphate	1.03	1.10
Choline chloride premix[1]	0.50	0.50
Layer microingredient premix[2]	0.50	0.50
Iodized salt	0.29	0.25
DL methionine	0.17	0.19
Tallow	4.00	4.00
Calculated Analysis		
ME (Kcal/kg)	2750	2900
CP (%)	15.70	16.50
Calcium (%)	3.20	3.20
Available P (%)	0.43	0.43
Lysine (%)	0.75	0.79
Methionine (%)	0.40	0.43
TSAA (%)	0.65	0.69

[1] Provided choline chloride in the diet at a level of 100 mg/kg.

[2] Provided per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU; cholcalciferol, 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 40 IU; vitamin K, 2.0 mg; pantothenic acid, 14 mg; riboflavin, 6.5 mg; folacin, 1.0 mg; niacin, 40 mg; thiamine, 3.3 mg; pyridoxine, 6.0 mg; vitamin B12, 0.02 mg; biotin, 0.2 mg; iodine, 0.5 mg; Mn, 75 mg; Cu, 15 mg; Zn, 80 mg, Se, 0.1 mg; Fe, 100 mg.

Table 4-2. Production parameters at sexual maturity and laying patterns until 40 wk of age of broiler breeders fed a STD or CON diet from 21 wk of age.

	Feeding Regimen[1]		
Parameter	STD	CON	SEM
	(Mean)		
BW at sexual maturity(g)	2753	2816	52
Days from photostimulation to first egg (d)	31.0	28.7	1.5
Days to consecutive >52 g eggs (d)	46.1	40.2	2.5
Weight of first egg (g)	45.7	44.9	1.0
Egg production traits[2]			
Total eggs	91.3	91.9	2.7
Settable eggs	86.0	83.3	2.6
Sequence analysis			
Prime sequence length (d)	11.66	11.03	1.59
Mean sequence length (d)[3]	4.07	3.64	0.40
Mean weekly sequence length[4]			
Early weekly mean length (d)	7.24	7.20	0.18
Late weekly mean length (d)	4.84^a	4.09^b	0.17
Overall mean length (d)	5.97^a	5.63^b	0.09
Pause analysis			
Mean pause length (d)[3]	1.15	1.23	0.06
Mean weekly pause length[4]			
Early weekly mean length (d)	1.87^a	1.54^b	0.11
Late weekly mean length (d)	1.45^b	1.72^a	0.09
Overall mean length (d)	1.62	1.65	0.05

[a-b] Means within a row with no common superscript differ significantly ($P<0.05$).

[1] STD = standard feed restriction; CON = concentrated feed fed at STD level.

[2] Egg production traits for the 16 wk period ending at 40 wk of age.

[3] Mean length calculated as mean of all sequences or pauses occurring in each bird

[4] Values weighted using weekly means. Sequences or pauses spanning more than 1 wk appear in two (or more) consecutive weeks. Early lay = 24 to 32 wk of age; Late lay = 32 to 40 wk of age; Overall lay = 24 to 40 wk of age.

Table 4-3. Plasma hormone and lipid parameters at 40 wk of age of broiler breeders fed a STD or CON diet from 21 wk of age.

Parameter	Feeding Regimen[1] STD	Feeding Regimen[1] CON	SEM
	(Mean)	(Mean)	
Plasma traits			
Estradiol-17β concentration(pg/mL)	110.3	106.7	12.0
Luteinizing hormone concentration (pg/mL)	2267	2696	242
Plasma lipid concentration (mg/mL)	29.10	27.06	3.10
Plasma VLDL			
VLDL lipid concentration (mg/mL)	23.11	20.52	2.70
VLDL triglyceride concentration (mg/mL)	15.21	13.14	2.91
VLDL phospholipid concentration (mg/mL)	6.56	6.40	0.83
TG:PL ratio	2.53	2.17	0.18

[a-b] Means within a row with no common superscript differ significantly ($P<0.05$).

[1] STD = standard feed restriction; CON = concentrated feed fed at STD level.

Table 4-4. Body weight, breast muscle, abdominal fatpad, and liver measurements at processing in 42 wk old broiler breeders with blank or estradiol-17β silastic implants and fed a standard or concentrated diet from 21 wk of age

		Breast Muscle		Abdominal fatpad		Liver		
Source	BW at processing	Weight	Percentage[1]	Weight	Percentage[1]	Weight	Percentage[1]	Liver lipid content[2]
	(g)	(g)	(%)	(g)	(%)	(g)	(%)	(%)
Feed[3]								
STD	3783[b]	626.6	16.48	196.6	5.20	65.8[b]	1.74	11.45[b]
CON	3958[a]	630.6	15.89	190.5	4.83	78.7[a]	1.99	16.53[a]
SEM	61	25.6	0.45	8.5	0.20	4.4	0.11	1.09
Hormone[4]								
BL	3826	633.6	16.45	190.3	4.98	69.1	1.81	12.81
E2	3913	623.6	15.93	196.7	5.05	75.5	1.92	15.17
SEM	29	25.6	0.45	8.5	0.20	4.4	0.11	1.09
	Probability							
Source of variation								
Feed	0.048	0.91	0.37	0.62	0.21	0.047	0.11	0.003
Hormone	0.32	0.78	0.42	0.60	0.81	0.31	0.43	0.14
Feed X Hormone	0.83	0.39	0.23	0.89	0.94	0.65	0.59	0.82

[a-b] Means within a column and within a main effect with no common superscript differ significantly.

[1] Percentage = tissue weight / BW X 100.

[2] Lipid percentage = liver lipid weight / liver weight X 100.

[3] STD = standard feed restriction; CON = concentrated feed fed at STD level.

[4] Hormone Implants: BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 4-5. Oviduct, ovary, and stroma weights at processing in 42 wk old broiler breeders with blank or estradiol-17β silastic implants and fed a standard or concentrated diet from 21 wk of age

	Oviduct		Ovary		Initial stroma[2]		Bare stroma[3]	
Source	Weight	Percentage[1]	Weight	Percentage	Weight	Percentage	Weight	Percentage
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Feed[4]								
STD	72.5	1.92	68.6	1.82	10.15	0.268	5.40	0.143
CON	72.4	1.84	72.9	1.85	9.53	0.241	5.30	0.133
SEM	2.5	0.08	3.5	0.09	0.45	0.011	0.23	0.005
Hormone[5]								
BL	71.5	1.87	69.9	1.83	9.55	0.250	5.25	0.137
E2	73.3	1.89	71.6	1.84	10.12	0.259	5.41	0.138
SEM	2.5	0.08	3.5	0.09	0.45	0.011	0.23	0.005
	Probability							
Source of variation								
Feed	0.99	0.46	0.39	0.79	0.34	0.10	0.69	0.19
Hormone	0.61	0.86	0.74	0.97	0.38	0.58	0.63	0.91
Feed X hormone	0.24	0.34	0.67	0.57	0.78	0.71	0.91	0.82

[a-b] Means within a column and within a main effect with no common superscript differ significantly.

[1] Percentage = tissue weight / BW X 100.

[2] Initial stroma = ovary without the large yellow follicles (follicles >10mm diameter removed).

[3] Bare stroma = initial stroma without the small yellow follicles or large white follicles (follicles >2mm diameter removed).

[4] STD = standard feed restriction; CON = concentrated feed fed at STD level.

[5] Hormone Implants: BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 4-6. Ovarian follicle numbers, small follicle atresia, mean large yellow follicle weight, and hierarchy size of broiler breeders at processing in 42 wk old broiler breeders with blank or estradiol-17β silastic implants and fed a standard or concentrated diet from 21 wk of age

Source	Ovarian follicles[1]					LYF parameters		
	LYF number	SYF number	LWF number	MWF number	Small atretic follicles[2]	Mean weight	Hierarchy positions[3]	Number of hierarchies[4]
	(#)				(#)	(g)	(#)	(#)
Feed[5]								
STD	5.94	15.20	16.96	31.29	45.19	9.71	5.94	1.00[b]
CON	5.97	12.73	15.80	32.75	43.98	10.52	5.59	1.07[a]
SEM	0.24	1.17	1.38	2.08	3.88	0.39	0.22	0.02
Hormone[6]								
BL	5.72	13.48	16.24	30.94	41.04	10.47	5.65	1.01
E2	6.19	14.45	16.52	33.11	48.12	9.75	5.87	1.06
SEM	0.24	1.17	1.38	2.08	3.88	0.39	0.22	0.02
	Probability							
Source of variation								
Feed	0.92	0.15	0.56	0.63	0.82	0.15	0.26	0.028
Hormone	0.18	0.57	0.89	0.47	0.20	0.20	0.47	0.14
Feed X Hormone	0.33	0.52	0.42	0.82	0.79	0.61	0.75	0.14

[a-b] Means within a column and within a main effect with no common superscript differ significantly.

[1] LYF = large yellow follicles (>10mm diameter); SYF = small yellow follicles (5-10mm diameter); LWF = large white follicles (2-5mm diameter).

[2] Number of atretic follicles < 5 mm in diameter.

[3] Number of follicle groups within 1 g.

[4] Hierarchies calculated as LYF divided by positions.

[5] STD = standard feed restriction; CON = concentrated feed fed at STD level.

[6] Hormone Implants: BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 4-7. Hatchability of settable eggs, egg weight, transfer weight, and chick weight and proportion of egg weight during the 2 wk implantation period (40 to 42 wk of age) in broiler breeders with blank or estradiol-17β silastic implants and fed a standard or concentrated diet from 21 wk of age

		Egg wt			Chick size	
Source	Hatchability	40 to 42 wk of age	Difference[1]	Transfer wt.	Wt	Percent of egg wt
	(%)	(g)	(g)	(g)	(g)	(%)
Feed[2]						
STD	86.0	65.8^{b}	0.43	58.7^{b}	47.0^{b}	70.7^{b}
CON	86.5	67.7^{a}	0.61	60.5^{a}	49.2^{a}	72.7^{a}
SEM	3.5	0.5	0.22	0.5	0.5	0.3
Hormone[3]						
BL	83.5	67.4^{a}	0.71	59.9	48.1	71.0^{b}
E2	89.0	66.1^{b}	0.34	59.3	48.1	72.4^{a}
SEM	3.5	0.5	0.22	0.5	0.5	0.3
	Probability					
Source of variation						
Feed	0.91	0.004	0.59	0.018	0.002	0.0001
Hormone	0.27	0.047	0.19	0.43	0.90	0.001
Feed X hormone	0.055	0.34	0.46	0.22	0.43	0.003

[a-b] Means within a column and within a main effect with no common superscript differ significantly.

[1] Difference in hormone implant period egg wt (40 to 42 wk of age) and egg wt in previous 2 wk period (38 to 40 wk of age).

[2] STD = standard feed restriction; CON = concentrated feed fed at STD level.

[3] Hormone Implants: BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 4-8. Plasma estradiol-17β and Luteinizing hormone concentration, plasma lipid content, and very low density lipoprotein lipid content, triglyceride (TG) and phospholipid (PL) content, and TG:PL ratio at processing in 42 wk old broiler breeders with blank or estradiol-17β silastic implants and fed a standard or concentrated diet from 21 wk of age

				VLDL traits			
Source	Estradiol-17β[1] concentration	LH[1] concentration	Plasma lipid concentration	Lipid concentration	TG concentration	PL concentration	TG:PL ratio
	(pg/mL)		(mg/mL)				
Feed[2]							
STD	135.9	2106	29.9	21.9	14.83	5.94	2.51
CON	123.6	2435	33.7	25.1	16.60	7.18	2.36
SEM	6.4	166	3.0	2.6	1.84	0.67	0.16
Hormone[3]							
BL	113.2[b]	2328	31.6	23.5	15.77	6.51	2.47
E2	146.1[a]	2213	32.0	23.5	15.67	6.61	2.41
SEM	6.4	166	3.0	2.6	1.84	0.67	0.16
	Probability						
Source of variation							
Feed	0.18	0.35	0.38	0.39	0.50	0.20	0.51
Hormone	0.002	0.74	0.92	0.99	0.97	0.92	0.80
Feed X hormone	0.56	0.63	0.94	0.87	0.95	0.61	0.61

[a-b] Means within a column and within a main effect with no common superscript differ significantly.

[1] Mean hormone concentration from d 7 and d 14 samples of hormone implantation period.

[2] STD = standard feed restriction; CON = concentrated feed fed at STD level.

[3] BL = blank silastic implant; E2 = estradiol-17β silastic implant.

Table 4-9. Mean values (± SEM) of plasma estradiol-17β, plasma lipid parameters, carcass traits, and ovarian morphology at 42 wk of age in 100% of hens, the upper and lower 50% of hens, and the upper and lower 25% of hens sorted by the processing VLDL-TG:PL ratio.

	Sorted by: Processing VLDL-TG:PL ratio				
Variable	100% of flock	Upper 50% of flock	Lower 50% of flock	Upper 25% of flock	Lower 25% of flock
Plasma estradiol-17β (pg/mL)	129.6 ± 7.4	131.3 ± 10.5	127.9 ± 10.5	135.2 ± 13.8	119.4 ± 13.8
VLDL parameters					
TG:PL ratio	2.44 ± 0.06	2.95 ± 0.08^{a}	1.94 ± 0.08^{b}	3.20 ± 0.09^{a}	1.75 ± 0.09^{b}
TG:PL ratio difference	0.08 ± 0.14	0.64 ± 0.20^{a}	-0.47 ± 0.20^{b}	0.95 ± 0.29^{a}	-0.65 ± 0.29^{b}
VLDL concentration (mg/mL)	23.5 ± 1.7	25.7 ± 2.4	21.2 ± 2.4	26.3 ± 3.5	20.2 ± 3.5
BW (g)	3868 ± 47	3834 ± 66	3901 ± 66	3823 ± 94	3843 ± 94
Liver wt. (g)	72.4 ± 3.2	68.2 ± 4.5	76.6 ± 4.5	65.2 ± 4.5	67.7 ± 4.5
Abdominal fatpad wt.					
(g)	193.5 ± 5.5	204.1 ± 7.8	182.9 ± 7.8	209.4 ± 10.5	178.6 ± 10.5
(% of BW)	5.01 ± 0.12	5.33 ± 0.18^{a}	4.68 ± 0.18^{b}	5.49 ± 0.28	4.68 ± 0.28
Ovary wt.					
(g)	70.8 ± 2.2	66.1 ± 3.1^{b}	75.5 ± 3.1^{a}	68.9 ± 4.4	73.9 ± 4.4
(% of BW)	1.84 ± 0.05	1.73 ± 0.08	1.94 ± 0.08	1.80 ± 0.10	1.91 ± 0.10
Mean LYF wt. (g)	10.25 ± 0.26	9.70 ± 0.37^{b}	10.79 ± 0.37^{a}	9.58 ± 0.46	10.38 ± 0.46
Estimated mean LWF wt. (mg)[1]	28.2 ± 1.9	21.4 ± 2.7^{b}	34.9 ± 2.7^{a}	20.6 ± 4.4^{b}	37.5 ± 4.4^{a}
Ovarian stroma[2]	7.16 ± 0.21	6.64 ± 0.29^{b}	7.67 ± 0.29^{a}	6.48 ± 0.52	7.70 ± 0.52
Atretic follicle number					
Large (>5 mm diameter)	1.33 ± 0.25	0.93 ± 0.36	1.73 ± 0.36	0.50 ± 0.56	2.13 ± 0.56
Small (<5 mm diameter)	44.8 ± 2.5	39.0 ± 3.6^{b}	50.7 ± 3.6^{a}	35.1 ± 5.2^{b}	53.4 ± 5.2^{a}

[a-b] Means within the upper and lower 50% of the flock, or within the upper and lower 25% of the flock with different superscripts are significantly different.

[1] LWF weight calculated as: (stroma without follicles > 5 mm diameter - stroma without follicles > 2 mm diameter) / number of LWF.

[2] Stroma without LYF and SYF.

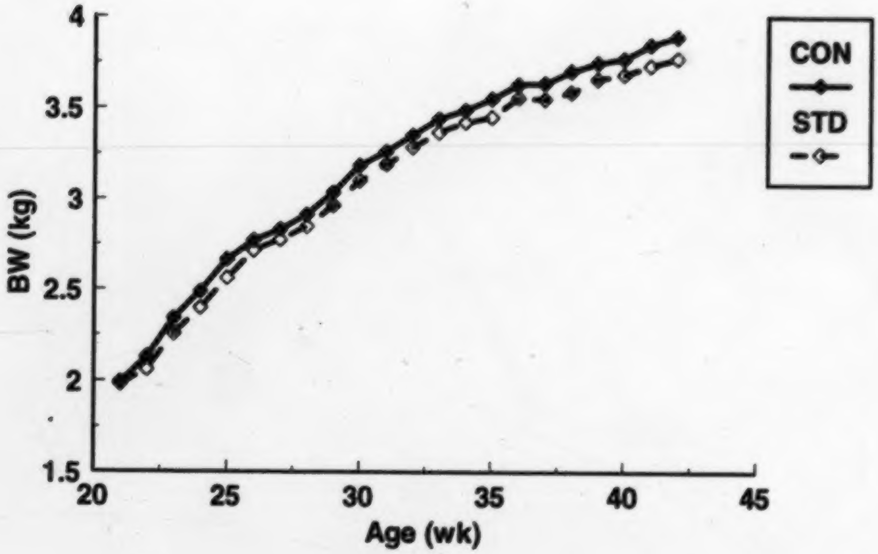

Figure 4-1. Body weight curve of broiler breeders on a standard (STD) or a concentrated (CON) feeding regimen beginning at 21 wk of age.

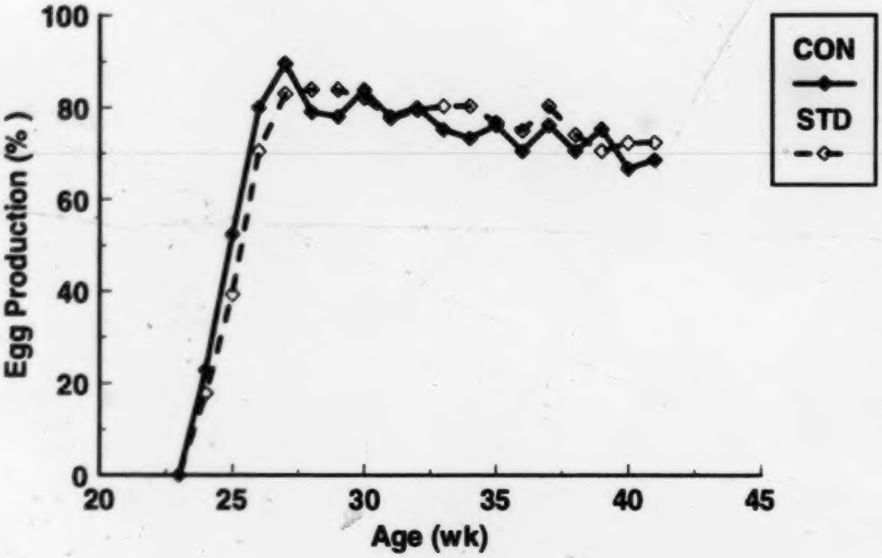

Figure 4-2. Effects of a standard (STD) or a concentrated (CON) feeding regimen on weekly hen-day egg production between onset of lay and 42 wk of age.

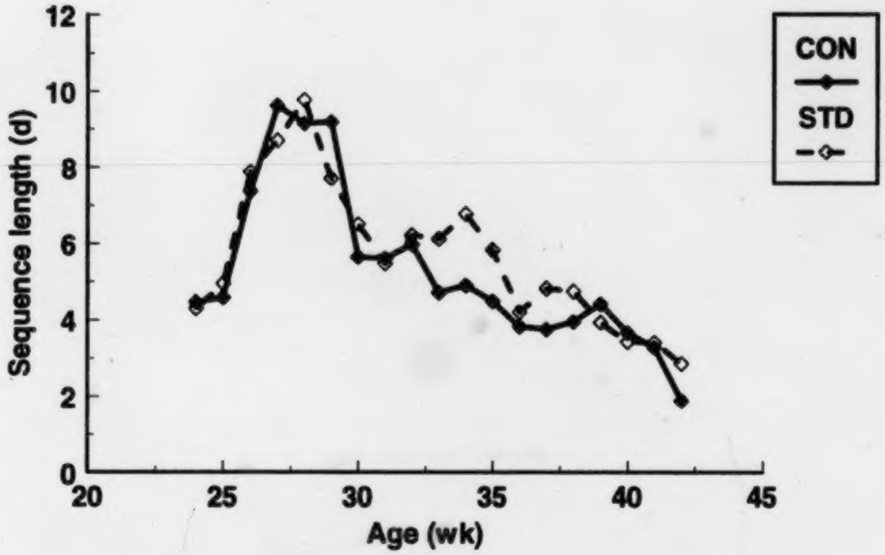

Figure 4-3. Mean weekly sequence length in broiler breeders on a standard (STD) or a concentrated (CON) feeding regimen from 21 to 42 wk of age.

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5. EFFECTS OF BODY WEIGHT AND FEEDING LEVEL FROM PHOTOSTIMULATION TO SEXUAL MATURITY ON CARCASS TRAITS, OVARIAN MORPHOLOGY, AND PLASMA HORMONE PROFILES IN BROILER BREEDER HENS

5.1 INTRODUCTION

Broiler breeder pullets are routinely restricted fed from an early age to reduce reproductive problems relating to selection for growth. The ovaries of growth-selected poultry strains are sensitive to overfeeding during reproductive development. As the birds are approaching sexual maturity (SM), overfeeding will result in the production of excess large yellow ovarian follicles (LYF), which will be more likely to be arranged in multiple hierarchies of large follicles (Hocking *et al.*, 1987, 1989; Katanbaf *et al.*, 1989; Yu *et al.*, 1992a). A multiple hierarchy results in increased production of unsettable eggs. The adverse effects of overfeeding are most prevalent prior to peak egg production (Yu *et al.*, 1992a). By 44 wk of age, overfeeding stimulates excess follicular recruitment to a lesser degree than at SM (Robinson *et al.*, 1993), and by 54 wk of age, ovarian morphology appears to be insensitive to moderate overfeeding (McGovern *et al.*, 1997).

During reproductive development, the number of LYF may be sensitive to timing of SM, feeding level, and BW. Early maturing birds have been found to have increased LYF numbers (Hocking *et al.*, 1988) and multiple LYF hierarchies (Hocking, 1992a; Renema *et al.*, 1997a) compared to later maturing birds. However, the primary effector of LYF numbers appears to be BW (Hocking and Whitehead, 1990; Hocking, 1993, 1996). A higher feeding level will elevate LYF numbers in birds of similar BW (Hocking, 1993), and will accelerate the sexual maturation process (Wilson and Harms, 1986; Yu *et al.*, 1992a). Light birds in a flock have been found to commence lay later and lay fewer eggs than medium or high-weight hens (Robinson and Robinson, 1991). As the attainment of SM may be influenced by BW (Brody *et al.*, 1980, 1984) and body fat (Bornstein *et al.*, 1984), monitoring changes in carcass parameters between photostimulation (PS) and SM in birds varying in BW may yield specific information on the reproductive disadvantage of birds with a small BW at PS. In this experiment, the effects of broiler breeder body size on carcass traits and ovarian morphology were examined at PS and SM in standard and naturally low or high weight birds. The interaction of *ad libitum* feeding with the variation in BW was also examined to compare the influence of excess nutrient availability with restricted feeding conditions and their effects on the relationship between nutrition and reproduction. Hormone profiles were constructed for estradiol-17β, luteinizing hormone (LH), and follicle stimulating hormone (FSH) to examine the effects of feed and body size on the profiles and their potential relationship with ovarian morphology and carcass composition at SM.

5.2 MATERIALS AND METHODS

Stocks and Management

A flock of 420 Shaver Starbro[1] broiler breeder pullets were reared in six 4.75 m X 5.85 m floor pens in a light-tight facility. Birds were allowed *ad libitum* access to water and reared following the breeder recommended target BW curve. A starter diet was fed from hatch to 3 wk of age, followed by a grower diet from 3 to 21 wk of age, and a breeder diet from 20 wk of age until processing (Table 5-1). All diets were fed in a mash form. Following 2 wk of *ad libitum* feeding, Skip-a-day feeding was used for the duration of the rearing period. Body weight was monitored by individual BW at 4 wk intervals and group weights were taken all other weeks. Pullets received 24 h of light (24L:0D) for the first 24 h, which was decreased to 8 h of light per d (8L:16D) until PS.

At 20 wk of age, birds were individually weighed and the data sorted by BW. Thirty birds near the pen mean were selected for the standard (STD) BW group representing target BW birds. An additional 30 birds were selected from both the top and bottom of the BW distribution to represent birds naturally either 20% lighter (LOW) or 20% heavier (HIGH) than the STD birds. At PS (21 wk of age), ten birds were randomly selected from each size group and processed immediately. These birds were used to examine effect of body size on carcass traits and to assess the degree of reproductive development at PS. Birds were euthanized by cervical dislocation. The breast muscle, liver, abdominal fatpad, oviduct, and ovary were dissected and weighed. Organs and tissues were returned to the carcass, which was stored at -15°C until carcass composition analysis was performed.

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[1] Shaver Poultry Breeding Farms Ltd., Box 400, Cambridge, ON, N1R 5V9

The remaining 20 birds within each size group were randomly assigned to either a standard restricted feeding regimen (RF), or an *ad libitum* feeding program (AL), resulting in a 2 X 3 factorial experimental design with feed (AL and RF) and size (LOW, STD, and HIGH) as the main effects. Birds were randomly assigned to individually cages and photostimulated by increasing day length to 13L:11D, followed by a 1 hr increase in light after 1 wk to 14L:10D. The feed of the RF-STD birds was allocated to maintain birds on the breeder recommended target BW curve and feed for the LOW and HIGH RF birds was allocated to maintain similar rates of BW gain to the STD birds (Table 5-2). Feed increases of 4 g or more were divided up into two to three smaller increases per week. The RF birds were fed individually on a daily basis and all birds were individually weighed at weekly intervals. Feed consumption of AL birds was recorded twice per week and daily feed intakes calculated. Total feed intake between 21 and 23 wk of age and between 21 wk of age and SM (defined as first oviposition) was calculated for all birds. The experimental protocol was approved by the Animal Policy and Welfare Committee of the Faculty of Agriculture, Forestry and Home Economics of the University of Alberta.

Carcass Traits at Sexual Maturity

Birds were maintained on assigned feeding regimens until first oviposition. At this time BW was recorded, and a blood sample was taken at 15:00 hr. The bird was euthanized the following morning at 8:00 hr. Processing BW was recorded and the breast muscle (*Pectoralis major* and *minor*), liver, abdominal fatpad (including fat adhering to the gizzard), oviduct, and ovary were removed and weighed. The liver was then stored at -15°C and the plasma at -30°C until lipids were quantified. The length of the shank (measured from the top of the hock joint to the footpad of the second toe) was recorded as an assessment of frame size. The number and weight of normal LYF (>10 mm diameter), and the number of small yellow follicles (SYF) (5-10 mm diameter), large white follicles (LWF) (3-5 mm diameter), and medium white follicles (MWF) (1-3 mm diameter) were recorded. Follicle size classifications were based on previous reports (Robinson and Etches, 1986), although in the current study the LWF range was continuous with the SYF and MWF denoted follicles 1-3 mm in diameter. Stroma weight was recorded with the LYF removed (initial stroma) and again when the SYF, LWF, and MWF had been removed (bare stroma) to assess treatment effects on large and small follicle types separately.

An assessment of the potential for multiple ovulations to occur was determined by assigning LYF of similar size (differing by less than 1 g or 1 mm diameter) to the same position in the hierarchy as reported previously (Renema *et al.*, 1995). Total number of positions and proportion of follicles in a multiple hierarchical arrangement were recorded. Number of complete hierarchies of LYF was calculated by dividing LYF number by the number of positions in the hierarchy. The number of post-ovulatory follicles (POF) on the stroma was recorded. Unexplained ovulations, defined as ovulations occurring prior to first oviposition, were calculated by subtracting any eggs previously laid or in the oviduct from the number of POF found at processing. The incidence of internal ovulation (as evidenced by yolk residue in the body cavity) was assessed. Follicles with a discolored and/or shrunken appearance were considered atretic (Gilbert *et al.*, 1983). The incidence of follicular atresia of the yellow follicles (> 5 mm diameter) and of the small follicles (< 5 mm diameter) was assessed by counting all visible atretic follicles in the appropriate ranges.

Dissected organs (except livers) were returned to the carcass following processing and stored at -15°C until carcass composition analysis was performed. The thawed carcasses were pressure-cooked for 4 h, and homogenized in a large industrial blender, and duplicate 150 g homogenate samples taken and freeze dried for 7 d, as described by Yu *et al.* (1990).

Corrections were made for moisture loss during carcass homogenization and freeze drying. Dried samples were homogenized in a feed grinder. Carcass samples were analyzed in duplicate for determination of total carcass dry matter, crude protein, lipid, and ash using standard chemical analysis procedures (AOAC, 1980). The livers were freeze dried, ground, and the total lipid content determined by petroleum ether extraction. True liver lipid content was calculated by adjusting recorded values to account for moisture loss during the tissue drying process. Total plasma lipid weight was determined by Folch extraction as described by Renema *et al.* (1997b).

Plasma Hormone Analysis

Beginning at PS, all birds were blood sampled at 3 d intervals to monitor changes in plasma estradiol-17β, luteinizing hormone (LH), and follicle stimulating hormone (FSH) concentrations. Blood samples were taken from the brachial vein using EDTA-coated vacuum blood collection tubes. Blood was centrifuged at 1500 *g* for 20 min at 3°C and stored at -30°C until analyses were performed.

Plasma estradiol-17β concentration was determined by RIA[2] as described by Renema *et al.* (1997b) in four assays. The inter-assay coefficient of variation was 5.89% and the intra-assay coefficient of variation was 3.69%. The LH and FSH determinations were performed using the methods of Krishnan *et al.* (1994) and Krishnan *et al.* (1993), respectively. The inter- and intra-assay coefficients of variation for the LH assays were 10.51% and 10.47%, respectively, whereas for the FSH assay they were 24.55% and 17.81%, respectively.

Statistical Analysis

Data collected from birds at PS were evaluated with one-way analysis of variance procedures of SAS® (SAS Institute, 1994). Source of variation for the parameters measured in birds processed at photostimulation was the size groups. The main effects within the 2 X 3 factorial design, Size and Feed, were applied to cages in a completely randomized design. Data collected after PS were evaluated by two-way analyses of variance using the General Linear

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[2] Kit Number TKE25, Diagnostic Products Corp., Los Angeles, CA 90045-5597

Models (GLM) procedures of SAS® (SAS Institute, 1994). Sources of variation for initial and final plasma traits, and for carcass parameters at SM were feeding regimen, body size, and the interaction of feed X size. Means were evaluated with Fisher's protected LSD procedure (Peterson, 1985). Differences between means were determined using the least significant difference procedure. The error variation for all variables consisted of the variation between birds within the interaction. Means within the interactions were compared only within a feeding regimen.

Plasma hormone profiles were compared using Kolmogorov-Smirnoff curve shape analysis (SAS Institute, 1994). Stepwise regression analysis was used to evaluate the relationship of processing and peak estradiol-17β concentrations with ovarian morphology and carcass parameters using $P < 0.15$ as the limit for inclusion. Pearson correlation coefficients (Steel and Torrie, 1980) were computed between reproductive, carcass, and plasma parameters within the AL and RF feeding regimens. One bird in the RF-L group died and as a result SEM values were based in the main effect or interaction group with the fewest birds. Unless otherwise stated, all statements of significance were based on testing at the $P < 0.05$ level.

5.3 RESULTS AND DISCUSSION

Carcass Morphology at Photostimulation (21 wk of age)

Carcass characteristics and composition traits of birds of the different body size groups are presented in Table 5-3 and Figure 5-1. Significant differences existed in BW between LOW and HIGH birds at 4 wk of age, and between all body size groups beginning 8 wk of age. The STD bird BW at 21 wk of age was 1995 g, with the LOW and HIGH birds weighing 18% less and 20% more, respectively.

With differences in BW, there were associated differences in carcass measurements (Table 5-3). Shank length was reduced in LOW birds compared to STD and HIGH birds (100 *vs.* 103 and 105 mm, respectively). Breast muscle and abdominal fatpad weights differed between size groups, with the greatest values obtained from the HIGH birds, and the lowest values from the LOW birds. On a percentage basis, both breast muscle and abdominal fatpad content was greater in STD and HIGH birds than in LOW birds. Together with the shank length data, this suggests that LOW bird growth may have been stunted and that they had inferior fleshing compared to STD and HIGH birds. In the current experiment, liver weight of LOW and STD birds (38.1 and 32.0 g, respectively) differed, but were both lower than that of HIGH birds (52.1 g). Early development of the reproductive tract (oviduct and ovary) was similar on a percentage of BW basis. However, on an absolute basis, oviduct weight was lower in LOW birds (0.44 g) than in HIGH birds (1.21 g) and ovary weight was lower in LOW birds (0.59 g) than in STD (0.93 g) and HIGH birds (1.00 g). The size of the reproductive tracts of STD and HIGH birds indicates that they may be at a more advanced developmental level than the reproductive tracts of the LOW birds. In *ad libitum* fed birds, evidence of follicular activity can occur as early as 14 wk of age (Hocking *et al.*, 1989), and by 18 wk of age, the ovaries of 25% can already contain LYF (Yu *et al.*, 1992a). Whereas oviduct and ovary weight did not correlate with BW within the body size groups of the current experiment, they did correlate across size groups (oviduct: r=0.462; P=0.010, ovary: r=0.512; P=0.004), which supports the theory that the reproductive tracts of the heaviest birds were more developed at this time. Within the HIGH body size group, ovary weight was correlated with fatpad weight (r=0.837; P=0.003). Body size-based differences in shank length, fatpad size and reproductive organs were similar to those observed in birds restricted fed to various levels (Fattori *et al.*, 1993), with LOW birds being represented by the most restricted treatments and HIGH birds by the least.

The total carcass protein, lipid, ash, and water content on an absolute basis increased significantly between the LOW, STD, and HIGH group birds, respectively (Table 5-3), reflecting the BW differences between these groups. On a relative basis, however, the carcass protein (mean = 20.4%) and ash content (mean = 3.33%) were very similar between groups. The LOW birds had a lower lipid content (6.3% *vs.* 9.5% on average) and a higher water content (70.0% *vs.* 66.3% on average) than the STD and HIGH birds. Relative carcass lipid content was negatively correlated with water content (r=-0.892; P<0.0001). The carcass analysis data clearly shows that the composition of the LOW birds differed from the STD and HIGH birds with regard to carcass lipid content in particular. The LOW birds had reduced lipid stores compared to birds of the larger size groups. This may be indicative of a nutrient shortage which may also relate to the reduced size of the reproductive tract relative to the STD and HIGH birds.

Carcass Morphology at Sexual Maturity

The AL feeding regimen increased BW at SM by 751 g compared to RF bird BW (3599 *vs.* 2864 g for AL and RF birds, respectively) (Table 5-4). These BW are similar to previously published values for birds on AL and RF feeding regimens (Robinson *et al.*, 1991; Yu *et al.*, 1992b). The AL bird BW had increased by 85% over BW at PS compared with 46% for the RF birds. Whereas the initial range of BW in the different body size groups compared to STD birds was 20% lighter or heavier in LOW and HIGH birds, respectively, at SM this difference had diminished to 5.1% lighter and 4.1% heavier in LOW and HIGH birds, respectively. An initial difference of approximately 400 g compared to STD birds decreased to 150 g at SM. This improvement in BW uniformity was most apparent in RF birds in the interaction, where SM-BW was 2752, 2858, and 2981 g for LOW, STD, and HIGH birds, respectively. Overall, the LOW birds weighed significantly less than STD or HIGH birds at SM. In the interaction, the LOW birds were lighter than only the HIGH birds in either feeding regimen.

In a study of broiler breeders approximately 22% lighter or heavier than a medium weight group at PS, Robinson and Robinson (1991) found that at SM the BW range was 500 g compared to a 230 g range in the RF BW at SM in the current study (Table 5-4). The birds of Robinson and Robinson (1991) were approximately the same initial BW as those of the current study, but management differed in that they were photostimulated at 22 rather than 21 wk of age and all birds received the same feed allocation. Their birds reached SM 234 g heavier, on average, than birds in the current study, which is likely a result of the later date of PS. Feed was allocated in RF birds of the current study at levels which would maintain constant rates of gain in the three body size groups (Table 5-2). These birds had been reared in floor pens, where presumably the larger birds were consuming greater amounts of feed than the smaller birds. Allocating the same amount of feed to all birds after PS could result in the HIGH birds being overly restricted and the LOW birds being overfed. Although this may have the effect of shortening the time to SM in light birds and lengthening it in heavy birds, it appears to have had no affect on the birds of Robinson and Robinson (1991) compared to the differentially fed birds of the current study, as they all came into production at a similar time and BW.

The rate of gain and feed intake of all birds was compared at 23 wk of age, when all treatments still had comparable bird numbers (Table 5-4). The average daily gain of LOW and STD birds was higher than that of the HIGH birds. This difference was primarily due to differences in AL bird gains (87.6 g/d on average in LOW and STD birds *vs.* 75.5 g/d in HIGH birds). The average daily feed intake between PS and 23 wk of age was 154 g/d in LOW birds compared to an average of 167 g/d in the STD and HIGH birds. Gut capacity of the smaller LOW birds may be limiting to their voluntary feed intake at this age. In the 14 d following PS, the AL birds gained an average of 83.9 g/d compared to 18.7 g for RF birds (Table 5-4). The 118% greater daily feed intake of AL birds compared to RF birds (223.5 g/d *vs.* 102.6 g/d, respectively) was responsible for this 349% increase in average daily gain. Birds switched to an *ad libitum* feeding program may initially eat larger amounts of feed than birds having long-term *ad libitum* access to feed (Hocking, 1996; Renema *et al.*, 1997b), and within 4 d feed intake will decrease to a consistent level (Robinson *et al.*, 1993). The BW curves for the current experiment (Figure 5-2) support these observations. Whereas the three RF body size groups continue to grow at a similar and steady rate, the AL birds grow very rapidly initially and then begin to level off in time. Each treatment BW mean was significantly different from all other treatment means each week after PS.

The daily feed intakes between PS and SM were similar in birds of the different body size groups (Table 5-4). It appears that despite reduced feed intakes immediately following PS in LOW birds, BW ultimately did not affect their ability to eat. As birds of each size group gained weight at a similar rate between PS and SM, it may be that BW differences at PS were based on behavioral effects. In broiler breeder flocks reared as a group, aggressive birds are found to grow larger more quickly while passive birds remain smaller and under more severe restriction conditions due to lack of feed access (Petitte *et al.*, 1981). The BW spread of the different size groups of the current experiment grew larger until 16 wk of age (Figure 5-1), supporting the premise that eating behavior may have contributed to flock BW variability.

The total feed intake between PS and SM followed a similar pattern to total BW gain during this time period (Figure 5-2). The total gain was 83% greater in AL compared to RF birds whereas total feed intake was only 56% greater. Total feed intake was higher in LOW birds relative to STD and HIGH bird intakes. This difference was most apparent in the RF interaction, where the LOW birds consumed a total of 5259 g of feed between PS and SM compared to 4241 and 2989 g consumed by the STD and HIGH birds, respectively. As the average daily gains and feed intakes of the different size groups were similar, the difference in total feed intake is due to the time taken to reach SM.

The AL feeding regimen caused birds to reach SM at 25.3 d after PS on average compared to 38.9 d in RF birds (Table 5-5). Figure 5-3 is an additive plot of the proportion of AL and RF birds reaching SM. Following a similar rise to 23 d after PS, the AL curve increased very steeply to 85% at 28 d, when the curve slope shallowed and continued up at a rate parallel to the RF curve. The initial similarity of the curves may be due to 25% of the birds in both feeding regimens having already reached the necessary BW or body composition threshold for reproduction (Brody *et al.*, 1980, 1984) or possibility to these birds being the heaviest within these groups at this time. The birds at the end of the AL curve may not have achieved their minimum requirements for commencement of lay. In a study of birds with high and low BW thresholds for reproduction, Eitan and Soller (1993) found the low-line birds entered lay sooner and at a lower BW than the high-line birds. The differences were hypothesized to be due to a decreased photoperiodic drive in the high-line relative to the low-line birds. Presumably this reduced light sensitivity is slowing the sexual maturation process.

The RF curve in the current study represents birds with a wide variety of BW and carcass compositions that consumed less than half the feed of AL birds. This caused their curve to be much more shallow to allow many of the birds to continue to grow, but still remarkably linear in its shape. Bornstein and Lev's (1982) additive plots for birds coming into lay after *ad libitum* or restricted feeding from hatch both had a distinctive lengthening of the final portion of the curve as the last few stragglers continued to delay their onset of lay. Renema (Thesis: Chapter 3) also observed a substantial delay in late maturing birds within a uniform flock of broiler breeders. The uniformity in BW and/or composition in the AL treatment birds following commencement of *ad libitum* feeding conditions resulted in a steeper curve of birds entering production compared to RF birds (Figure 5-3) in much the same way delaying PS to a later age would. A later PS allows the birds to continue to grow, resulting in a much more uniform age at SM in both broiler breeders (Robinson *et al.*, 1996), and turkeys (Hocking, 1992b).

A greater initial body size was associated with a reduced age at SM (Table 5-5). In the additive plot of birds reaching SM, the HIGH and STD curves appear to be parallel and the LOW bird slope is reduced (Figure 5-3). Examination of the Feed X Size interaction plots shows the RF-HIGH birds reach SM in a similar pattern to that of AL birds whereas the RF-STD and -LOW birds both have an increased variability in reaching SM as indicated by the reduced slope of their curves.

The advantage of the early age at which birds fed *ad libitum* throughout rearing reach SM compared to restricted fed birds is believed to be nullified by their early small egg production (Hocking, 1996). In the current study, where *ad libitum* feeding was only used after PS, egg size did not differ between feeding regimens, but was greater in LOW body size birds than in STD and HIGH birds (Table 5-5). Although LOW bird eggs may reach settable weight more quickly than eggs of other body size groups, that would not likely make up for eggs lost due to the extra time needed to reach SM. Robinson and Robinson (1991), examined the effects of initial body size on subsequent egg production and found that besides commencing lay later, the low-weight birds also exhibit the poorest laying performance. A large body size did not result in the detrimental effects to egg production observed in *ad libitum*-fed hens (Robinson *et al.*, 1991), which was theorized to be due to an overly heavy hen not necessarily being an obese hen as carcass fat was 7.1% lower in heavy hens than in overfed hens (Robinson and Robinson, 1991).

Breast muscle, abdominal fatpad, and liver weights are presented in Table 5-6. Feeding regimen caused significant increases in the absolute weights of all of these tissues. As the AL birds weigh 735 g (25.7%) more than RF birds on average, these results are not surprising. Breast muscle weight was the most similar, with the AL breast muscle weighing only 9.6% more than the RF breast. As with the BW at SM, breast muscle weight of the LOW birds was lower than that of the STD and HIGH birds, although in the interaction, the breast muscle of the LOW birds was only significantly less than the HIGH birds within the AL and RF feeding regimens. The relative weight of breast muscle was greater in RF birds, where the breast represented 16.2% of their BW compared to 14.6% in AL birds. There was no difference between relative breast muscle weights among the body size groups. The abdominal fatpad weighed 124.0 g in AL birds, on average, which was 125% greater than the 55.0 g of fatpad present in RF birds. This large difference was also present in the relative abdominal fatpad weight, with AL fatpads being 3.68% of total BW compared to 1.96% in their RF counterparts. The abdominal fatpad weight of the HIGH birds was numerically elevated over STD and LOW bird fatpad weight (97.7 g *vs.* 85.4 g, on average, respectively) (P=0.16), due primarily to variation in AL bird values (P=0.77). The relative abdominal fatpad weights were very similar in all body size groups, however. Whereas the proportion of breast muscle was not different from PS values (Table 5-3), the proportion of abdominal fatpad weight had increased 2.2 to 5.3-fold over PS values. Abdominal fatpad size was similar to values reported by Hocking (1996) and about half that of values reported by Yu *et al.* (1992b).

The weight of the liver was greater in AL than in RF birds both on an absolute and a relative basis (Table 5-6). The AL livers weighed 86% more than RF livers, which is likely an effect of feed intake and nutrient processing in these birds whose average daily gain between PS and SM was 181% greater than the RF bird rate of gain (Table 5-4). Liver weight was significantly greater in HIGH birds than in LOW birds due to differences in the AL interaction, but the difference disappeared when liver weights were compared on a relative basis (Table 5-6).

The lipid content of the liver accounted in part for the liver weight difference. The AL bird livers contained 16.1 g of lipid compared to 3.0 g in RF bird livers. This 13.1 g difference represents 36% of the 36.5 g difference in liver weight between the feeding regimens. On a percentage basis, lipid accounted for 18.0% of AL bird livers compared to 6.6% of RF bird livers. There was a body size effect on relative liver lipid weight due to differences in AL birds. The lipid content of LOW bird livers was less then that of HIGH bird livers, as demonstrated by the interaction values (12.9% *vs.* 22.1% in AL-LOW *vs.* AL-HIGH birds, respectively). The liver weight of RF birds did not change between PS and SM. The liver lipid content ranged from 2.0 g in LOW birds to 2.6 g in HIGH birds at PS (data not shown) and from 2.1 g in LOW birds to 3.7 g in HIGH birds at SM. Despite increased rates of lipid synthesis to supply reproductive processes, no excess lipid had accumulated in the liver at this time. The significant lipid accumulation in AL bird livers at SM could be due to the rate of lipid synthesis in the liver exceeding the rate of clearance. Overfeeding chickens is believed to saturate the very low density lipoprotein (VLDL) plasma lipid carrier synthesis and transport system, resulting in liver accumulation of triglyceride (TG) (Leclercq *et al.*, 1974). If the production of TG by the liver surpass its ability to form VLDL, excess TG can be temporarily stored in cytoplasmic triglyceride-rich vesicles (TGRV) (Mooney and Lane, 1981). The half-life of plasma VLDL from chronically overfed birds has been reported to be increased as indicated by a slow turnover rate of the enlarged lipid pool of the liver (Bacon *et al.*, 1978). As more than 90% of fatty acid synthesis has been reported to occur in the poultry liver (Leveille *et al.*, 1975), the *ad libitum* feeding of birds can have substantial effects at the liver level. It is not known if the lipid accumulations in AL birds of the current experiment were due specifically to TGRV accumulation, or if they would be partly responsible for negative long-term reproductive implications associated with birds fed in this manner. Both liver weight and lipid content of birds fed *ad libitum* for 22 wk have been reported to be similar to those of restricted fed birds due to the feed intake of *ad libitum* fed birds becoming similar to that of restricted fed birds (Renema *et al.*, 1997b).

Carcass composition analysis, which includes absolute and relative measures of total carcass protein, lipid, ash, and water, is presented in Table 5-7. The weight of carcass protein, lipid, ash, and water were all significantly greater in AL than RF birds. Due to the AL bird BW being substantially higher than RF BW, the proportion of the carcass components is a more telling comparison of AL and RF birds. The proportion of protein, ash, and water were all greater in RF birds. Carcass lipid, however, remained significantly greater in AL compared to RF birds. Whereas the absolute weights of protein, ash, and water were increased by 12.2%, 7.6%, and 15.7%, respectively in AL birds, carcass lipid was 100.0% greater (740 *vs.* 370 g for AL and RF birds, respectively). On a percentage basis, this excess lipid represented 20.5% of AL bird BW compared to 12.8% for RF birds. The 7.7% extra lipid content of AL birds occurred primarily at the expense of water (5.0%), with the remainder being compensated for by protein (2.2%) and ash (0.5%). A strong inverse relationship between carcass lipid and water has been reported previously in broiler breeder hens (Robinson and Robinson, 1991; Robinson *et al.*, 1991). The difference in the carcass water to carcass fat ratio changed from 11.1, 7.4, and 6.6 in LOW, STD, and HIGH birds at PS, respectively, to 3.35, 3.66, and 3.31, respectively, at SM. The carcass water to lipid ratios differed significantly due to feeding regimen alone, with AL birds measuring 2.8, on average, compared to the 4.8 value calculated for RF birds ($P<0.0001$), indicating a high water to lipid content in RF birds compared to AL birds.

Hen size significantly increased carcass protein content, with the LOW, STD, and HIGH birds containing 590, 628 and 676 g of protein, respectively (Table 5-7). Differences of this magnitude were also present in the different bird sizes of the interaction. When compared on a percentage basis, the HIGH birds contained more protein (20.1%) than the LOW or STD birds (19.4 and 19.5%, respectively). The relative protein content of birds was not different at PS (Table 5-3). The protein content difference at SM suggests that a HIGH initial size may give an advantage with regard to protein deposition. The STD and HIGH birds both had higher water content than LOW birds, although on a percentage basis the LOW and STD birds had a higher water content than HIGH birds. The HIGH birds contained 59.2% water compared to 61.2% on average in the other size groups. The difference in the water content of HIGH birds is split primarily between carcass protein (0.7%) and carcass lipid (1.0%). The proportion of carcass lipids did not differ between size groups, however.

The absolute ash weight differed significantly between all size groups, with the LOW birds having the lowest ash content and the HIGH birds having the highest. As ash content can be used as an indicator of frame size, the reduced ash content in LOW birds suggests some degree of growth stunting occurred. Ash as a percentage of BW was not significantly different (P=0.16), but was numerically lower in the LOW size group compared to the HIGH birds. Other indicators of stunting were the reduced BW (Table 5-4) and breast muscle weight (Table 5-6) at SM compared to the STD and HIGH size group birds. The best indicator, however, was the reduced LOW bird shank length, which was 102.7, 105.4, and 105.5 mm in LOW, STD, and HIGH birds, respectively (P=0.010). Birds with a small body size may be similar to birds having undergone severe food restriction during the rearing period, which results in stunted growth (Brody *et al.*, 1980).

The abdominal fatpad weight is generally a good indicator of carcass fatness. As the most readily altered lipid depot in response to changing nutritional status, its size would be expected to closely relate to the bird's recent feeding program, especially since its size increased from 19.9 g at PS (Table 5-3) to 89.5 g (Table 5-6) on average at SM. Whereas abdominal fatpad weight correlated well with carcass lipid weight at PS (r=0.956; P<0.0001), at SM in RF birds this declines slightly (r=0.902; P<0.0001) and slightly more in AL birds (r=0.734; P<0.0001). The correlation of relative abdominal fatpad weight with relative carcass lipid weight, both of which are highly correlated with BW, is very strong in RF birds (r=0.884; P<0.0001), and weaker in AL birds (r=0.570; P=0.001) at SM. It is likely that in the *ad libitum* fed birds, fat is being deposited throughout the carcass and that other lipid depots may be being preferentially deposited to.

Brody *et al.* (1984), in their examination of the relationship between fatness and laying status, found that the fattest group did not follow the trends of the other groups, and concluded that if there is a body fat threshold for onset of lay, the fattest birds had surpassed it. The inferior correlation of abdominal to carcass lipids in the AL birds of the current experiment may also indicate that this group had surpassed any carcass lipid thresholds and were now behaving independently. The abdominal fatpad weight as a proportion of BW ranged between 3.5% (STD) and 3.8 % (HIGH) in AL birds and between 1.9% (LOW and STD) and 2.1% (HIGH) in RF birds (Table 5-6). Although both ranges represent very uniform relative abdominal fatpad weights, the difference between the AL and RF values supports the hypothesis that the AL birds have surpassed lipid thresholds. The range in carcass lipid content was even tighter, ranging between 20.1% (STD) to 20.9% in AL birds and between 12.1% (STD) and 13.6% (HIGH) in RF birds (Table 5-7). With a standard error of 0.8%, these values were tightly clustered within a specific range of values within birds on the AL or RF feeding regimen.

The attainment of SM is believed to be governed in part by BW (Brody *et al.*, 1980, 1984) and body fat (Brody *et al.*, 1984). Although age may also be important (Brody *et al.*, 1980, 1984), it is likely not a large factor in the current study. Ovaries of *ad libitum* fed birds can show signs of follicular activity by 14 wk of age (Hocking *et al.*, 1989), demonstrating that the hypothalamal-pituitary-gonadal axis is capable of functioning well before PS. By the time of SM in the current study, the large differences in carcass lipid content between birds of the different size classes at PS had disappeared (Tables 5-6 and 5-7). Whereas lipid levels within the AL and RF feeding regimen birds were very uniform, they may have been the result of the bird growing to a particular BW range or lean body mass. Soller *et al.* (1984) used quantitative feed restriction during rearing to examine minimum weight requirements for SM. They reported that fat content alone was not sufficient to initiate SM, but that there may instead be a lean body mass requirement as indicated by uniform lean BW and protein contents across treatments. Furthermore, in a study using Japanese quail, Zelenka *et al.* (1982) reported that birds on restricted or *ad libitum* feeding entered lay with variable proportions of abdominal fat, but similar breast muscle weights. Lean body mass is also more closely related to measures of ovarian development at SM, such as number of LYF, than measures which include carcass lipid (Hocking, 1993). Soller *et al.* (1984) concluded that body fat may still be important in determining the timing of the onset of lay, but that in *ad libitum* fed birds, where fat levels can be significantly elevated, birds must have reached this minimum fat percentage before they attained the minimum required lean BW. The relative carcass protein weights in the current experiment, although increased in HIGH birds relative to the other size groups, had a range in values similar to that of lipid weight, varying little around the means of 18.6% and 20.8% observed in the AL and RF groups, respectively (Table 5-7). The relative breast muscle weight, which can be an indicator of protein content, had a similarly low variation among size groups around a mean value of 14.6% in AL birds and 16.2% in RF birds (Table 5-6).

There was a 1021 g difference in BW at SM between the lightest group (RF-LOW) and the heaviest group (AL-HIGH) (Table 5-4). The protein content of these groups at SM was 569 g in RF-LOW birds, and 722 g in AL-HIGH birds (Table 5-7). When compared within RF birds, the RF-HIGH birds reached SM with 60 g more protein and 57 g more fat than the 230 g lighter RF-LOW birds. Based on these data it seems improbable that either the AL birds or the larger RF birds contained more fat because they were slow in achieving a critical protein content. Robinson and Robinson (1991), in comparing BW at SM in restricted fed low, medium, and high BW birds, found a 500 g difference between low and high-weight birds and theorized that the lower BW at SM in low BW birds may be due to a lower threshold for critical BW and/or body composition. With a naturally smaller build, this appears to be a reasonable conclusion to explain BW differences at SM among RF birds of the current experiment. An alternate explanation is that high BW birds have met and surpassed their threshold BW while waiting for a photostimulatory cue.

The daily gain of the AL birds between PS and SM was consistent between the different body size groups, ranging between 64 g/d in AL-LOW birds to 68 g/d in AL-STD and -HIGH birds (Table 5-4). Considering that these birds had *ad libitum* access to feed, and that the mean daily gain of RF birds was only 24 g/d compared to these birds, it is conceivable that they are growing at a maximal rate. The process of sexual development typically takes 3 to 4 wk in the chicken (Etches, 1993). The mean time to SM from PS in AL birds was 25.3 d (Table 5-5), which suggests sexual development was not held back at PS by a shortage in carcass protein, lipid, or BW. The 7.6 d numerical difference in day of SM between HIGH and LOW birds within the AL group may still be due to initial BW and carcass composition, however, as the LOW birds initially had a lower relative breast muscle weight, lower relative lipid content, and lower reproductive tract weight than the STD and HIGH birds (Table 5-3).

Reproductive Morphology at Sexual Maturity

The absolute weight of the oviduct did not differ due to feeding regimen or to body size, averaging 60.8 g in all birds (Table 5-8). As a result of its growth to a constant size in birds differing in BW (Table 5-4), the relative oviduct weight was greater in RF (2.2%) compared to AL (1.9%) feeding regimen birds and in LOW body size birds compared to STD and HIGH body size birds (Table 5-8). Ovary weight in AL birds was 38% greater than that of RF birds. This difference was also apparent in the relative ovary weight, which was 2.45% *vs.* 2.16% in AL and RF birds, respectively. Ovary weight did not differ due to body size and varied little within the AL and RF interactions. The initial stroma weight numerically differed between AL and RF feeding regimen birds on an absolute basis (8.63 *vs.* 7.54 g) (P=0.069). When the bare stroma weights of the AL and RF birds were compared (3.59 *vs.* 2.70 g), the difference was significant, indicating that overfeeding the birds may have caused differences in ovarian morphology in follicles that are prehierarchical. As the bare stroma contained follicles less than 1 mm in diameter, a heavier stroma weight may indicate greater numbers of these estradiol-17β producing follicles, thereby affecting the total estradiol-17β output of these ovaries. On a percentage basis, stroma weights did not differ due to feeding regimen or to body size. The difference in the proportion of initial stroma between LOW (0.30%) and HIGH (0.24%) body size groups approached significance (P=0.054), however, which was an effect of the reduced BW in LOW compared to HIGH birds at SM.

The difference in ovary weight between AL and RF birds at SM was due to the number of LYF (Table 5-9), which numbered 11.0 compared to 7.1 in AL and RF birds, respectively. Within the RF body size groups, there was a numerical reduction of 1.2 follicles in the RF-LOW birds compared to the other size groups (P=0.13). The number of LYF at SM is known to be reduced in birds needing a longer time period to reach SM (Hocking *et al.*, 1988). The number of LYF in AL and RF birds is in agreement with those of Hocking (1996) for similarly treated birds, but below the values of 11.7 and 9.3 reported by Yu *et al.* (1992a). Differences in LYF of the magnitude demonstrated here between AL and RF birds have been demonstrated to persist in some form through to 62 wk of age (Yu *et al.*, 1992a). There were no significant differences due to the main effects or the interaction for SYF, LWF, or MWF numbers. The number of MWF was 187 in AL birds compared to 161 in RF birds, which, although not significant (P=0.070), supports the premise that the higher bare stroma weight in AL birds may be due to an increased population of small follicles. Small follicle numbers were not related to LYF number, which concurs with the observations of Hocking *et al.* (1989) and Renema (Thesis: Chapter 3). Hocking (1996) theorized that the probability of recruitment to the LYF hierarchy may not be closely related to the growth of pre-hierarchical follicles and that these processes may be related to different underlying physiological processes.

Small atretic follicles numbered 10.3 in AL birds compared to 32.3 in RF birds (Table 5-10). The effect of body size was not significant (P=0.12), particularly because of the similarity in AL interaction values. In RF birds, however, small atretic follicles numbered 42.2, 30.6, and 24.0 for LOW, STD, and HIGH birds, respectively, with the RF-LOW value differing significantly from the RF-HIGH value. The number of small atretic follicles appeared to increase with time to SM. Although small atretic follicles were not correlated with day of SM in AL birds due to very low incidence (r=-0.076; P=0.69), this relationship was strong in RF birds (r=0.619; P=0.0003). As the initial stroma weight (r=0.443; P=0.016) and the bare stroma weight (r=0.622; P=0.0003) are both also increasing in weight with longer reproductive development time in RF birds, it appears that small follicle atresia is a method of controlling small follicle numbers. Renema (Thesis: Chapter 3) found that the rate of small follicle atresia was negatively correlated with the number of LYF on the ovary in the latter half of the flock reaching SM. In the current study these traits were numerically related in RF birds (r=-0.275; P=0.15), possibly because birds were not subdivided based on day of SM. The inferior egg production of the low-weight birds studied by Robinson and Robinson (1991) may be due in part to elevated rates of small follicle atresia. A naturally high incidence of small follicle atresia may be limiting the ovary's ability to generate an adequate number of LYF to maintain comparable rates of egg production to the larger bird groups.

Small follicle atresia was not a factor in the assembly of the LYF hierarchy of the AL birds due to their rapid onset of lay following PS. Renema (Thesis: Chapter 3), concluded that overfeeding during sexual maturation may cause ovarian development to proceed more quickly than the mechanisms controlling recruitment of follicles into the LYF hierarchy are able to be established, resulting in excess LYF production. If the physiological systems controlling reproductive function and LYF recruitment are continuing to develop as birds are coming into production, the elevated LYF numbers associated with a short time period between PS and SM could be avoided by extending this period. As the first birds into production tend to be the heaviest birds with a relatively high fat content, dividing the flock at PS by BW and feeding the heavy group at a lower level than the light group may be effective in delaying SM and reducing LYF numbers. Dividing pullets according to BW as part of a feed restriction program has been used effectively in broiler breeders to improve flock uniformity at PS (Petitte *et al.*, 1981), although this is not practiced commercially in North America.

Lean body mass has been reported to be related more precisely than BW to the number of ovarian LYF at SM (Hocking 1993, 1996). In the current experiment there was a positive relationship across all treatments between both BW and protein content with number of LYF because the much larger AL birds all had more LYF (Figure 5-8). A more meaningful comparison must be done within the feeding regimen, however, where BW was not correlated with number of LYF in either AL (P=0.83) or in RF (P=0.74) birds. Lean BW, calculated as the weight remaining after carcass lipid was subtracted, was also not correlated with number of LYF in either feeding regimen (P=0.46 and P=0.66 for AL and RF birds, respectively). The use of such a diverse group of initial body sizes and the associated staggered effect on timing of SM makes this type of comparison difficult because LYF numbers will have decreased in time (Hocking *et al.*, 1988) as both BW and protein content increased as birds continued to grow. In AL birds, lean BW as a percentage of BW had a numerical relationship with LYF number (r=0.325; P=0.079) and relative carcass protein weight in RF birds was correlated with LYF number (r= 0.376; P=0.044). In both feeding regimens, the proportion of protein in the carcass declined over time (r=-0.410; P=0.028) as carcass lipid increased (r=0.427; P=0.020), demonstrating that the relationship between measures of protein or lean content and LYF number are based on a time effect.

The stepwise regression performed within the AL and RF feeding regimens for the dependent variable, LYF number, revealed that primarily measures of ovarian size are associated with the number of LYF. In AL birds, ovary weight (P<0.0001) and the proportion of LYF in a multiple arrangement (P=0.009), both of which are directly related to LYF number, were the only significant variables introduced into the model. In RF birds, relative ovary weight (P<0.0001), small atretic follicle number (P=0.002), liver weight (P=0.012), MWF number (P=0.12), and initial stroma weight (P=0.10) were all entered into the model. Liver weight may be an indicator of lipid and yolk precursor synthesis potential in RF birds, where these mechanisms are not hidden by excessive rates of lipid metabolism as in AL birds. As with the late maturing birds of the study reported by Renema (Thesis: Chapter 3) the number of small atretic follicles on the ovary were inversely related to LYF number.

Unexplained POF, which represent ovulations not accounted for by eggs laid or by developing eggs in the oviduct, were greater in AL (2.13) than in RF (0.31) birds (Table 5-10). There was a trend towards reduced unexplained POF in LOW body size birds (P=0.11) due to significant differences within the AL body size groups, where the AL-LOW birds had fewer unexplained POF (1.50) than AL-HIGH birds (2.60) while AL-STD birds were intermediate (2.30). The incidence of unexplained POF have been reported to be higher in birds coming into production early (Renema *et al.*, 1997a), which tend to also have elevated LYF numbers (Hocking *et al.*, 1988). Follicles ovulated prior to first oviposition are presumably lost to processes such as internal ovulation. However, the incidence of internal ovulation was low in all treatments and no significant relationships were observed. Melnychuk *et al.* (1997) reported that increased incidence of unexplained POF in turkeys may be due to the ovary reaching a mature state prior to the oviduct, resulting in loss of potential eggs due to oviduct incompetence.

The weight of the egg and yolk increase as the bird ages, resulting in a higher yolk:albumen ratio (O'Sullivan *et al.*, 1991) and a larger hatch weight (Shanaway, 1984). Both egg weight (Table 5-5) and yolk weight (as represented by F1 weight) (Table 5-10) were greatest in the LOW body size birds. As these birds reached SM at the highest age, these results concur with expected patterns of increased egg and yolk weight with age. Robinson *et al.* (1996) found that despite significant increases in the weight of the largest follicle in groups of birds reaching SM progressively later, there was no relationship with the weight of the egg. In the current experiment, the comparison of yolk and egg weight values for AL and RF birds was also not in agreement. The LYF on the RF bird ovaries weighed more than those on the AL bird ovaries as indicated by their mean LYF weights (6.71 in AL birds *vs.* 7.39 in RF birds) (Table 5-10). Whereas the weight of the F1 follicle followed this pattern with its numerically higher weight in RF birds (P=0.13), egg weight was numerically greater in AL birds by 1.9 g (P=0.17). Renema *et al.* (1995) reported that egg size in restricted fed turkeys was reduced at the expense of albumen content, whereas yolk weight was not affected. Variability in egg weight relative to yolk weight in AL and RF birds is likely due to an increased albumen content in AL bird eggs.

The arrangement of LYF in the hierarchy was compromised in the AL birds due to their elevated LYF numbers. The 3.9 extra LYF on AL ovaries (Table 5-9) resulted in a higher number of follicles being in pairs or triplets of follicles of similar size (8.5 *vs.* 2.6 for AL and RF birds, respectively), representing 74.2% and 33.7% of LYF in AL and RF birds, respectively (Table 5-10). Increased pairing of follicles can disrupt normal ovarian function through increased unsettable egg production (Hocking *et al.*, 1987, 1989; Yu *et al.*, 1992a). The number of positions in the LYF hierarchy (groups of follicles differing by <1 g) related to the number of days in which at least one follicle was recruited (Hocking, 1996). The AL birds had 6.5 positions, which was 0.7 more than the RF birds had, on average, and indicates that the AL ovaries can maintain a higher number of follicles without pairing occurring. Despite this advantage, the AL birds had an average of 1.7 complete hierarchies of LYF on the ovary compared to 1.2 on the ovaries of RF birds. The hierarchy number represents the average number of follicles that can potentially ovulate on a given day. The AL-STD birds maintained 11.4 LYF compared to 10.3 LYF in the AL-HIGH birds (Table 5-9). However, due to a significantly greater number of hierarchy positions (7.0 *vs.* 6.2 for AL-STD *vs.* AL-HIGH birds, respectively) (Table 5-10), the number of hierarchies in these groups were identical (1.65). In 44 week old broiler breeders, Robinson *et al.* (1993) reported a 47% difference in birds demonstrating simultaneous follicular development following 2 wk of *ad libitum* or restricted feeding with no affect on LYF number. They concluded that the number of LYF maintained by the ovary may not be as important as the uniformity of spacing of the follicles in ensuring the more regular, single ovulation of follicles. If the AL birds of the current study had the same number of hierarchy positions as the RF birds did (5.8), the number of hierarchies they were maintaining would significantly increase to 1.9 from the 1.7 actually present, demonstrating the important role of LYF arrangement in ovarian control.

Plasma Lipid and Hormone Parameters

The plasma concentrations of estradiol-17β, LH, and FSH at PS and SM, and mean values of samples taken between PS and SM are presented in Table 5-11. At PS there was difference in estradiol-17β concentration due to body size, with HIGH bird plasma estradiol-17β measuring 49.1 pg/mL compared with a mean value of 35.5 pg/mL in the LOW and STD groups. This suggests that some initial maturation had occurred in the ovaries of HIGH birds prior to PS. Besides being the heaviest birds at PS, they also had the highest absolute abdominal fatpad and carcass lipid content (Table 5-3). Although no follicles over 1 mm in diameter were present at PS, the small white follicle number (<1 mm in diameter) may have been increased in HIGH birds as indicated by their greater bare stroma bare stroma weight compared to LOW birds (Table 5-3). Plasma concentrations of FSH and estradiol-17β at PS were strongly correlated ($r=0.522$; $P<0.0001$), which suggests that elevated estradiol-17β concentrations were due to increased follicle development.

The difference in plasma estradiol-17β concentration between body size groups at processing approached significance (P=0.062) (Table 5-11), with the increased HIGH bird concentrations over STD birds being influenced by significant differences between these size groups within the AL interaction. The mean plasma estradiol-17β concentration for the PS to SM time period was identical in AL and RF birds (113.4 pg/mL), but was greater in the HIGH birds compared to the STD and LOW birds (134.1 *vs.* 103.1 pg/mL on average, respectively). The plasma estradiol-17β profiles are presented in Figure 5-4, where the HIGH bird curve is separated from the STD and LOW bird curves. The estradiol-17β concentrations were elevated in HIGH birds despite similar small follicle numbers (Table 5-9) and bare stroma weights (Table 5-8) to the STD and LOW birds. The steroidogenic capacity of small white follicles (<1 mm) have been reported to be enhanced in response to increased feed intake (Yu *et al.*, 1992c). Although average feed intake values for this time period are confounded by time (Table 5-4), feed intake in LOW birds in the 2 wk directly following PS were lower than those of STD and HIGH birds. Mean plasma estradiol-17β concentrations differed the most between the LOW and HIGH birds within the RF feeding regimen (86.2 *vs.* 144.4 pg/mL, respectively) (Table 5-11). These values were inversely correlated with the number of small atretic follicles on the ovaries of RF birds (Table 5-10) (r=-0.491; P=0.008). Elevated mean plasma estradiol-17β concentrations in HIGH birds may be related to the relationship between age at SM and follicle management rather than to feeding level or body size.

The plasma LH and FSH concentrations at PS and SM did not differ due to feeding treatment or to body size (Table 5-11). Mean plasma LH concentration for the PS to SM period was greater in AL birds (4.40 ng/mL) than in RF birds (3.38 ng/mL). This can be seen clearly in the plasma LH profile (Figure 5-5), where LH concentrations in AL birds were greater than RF birds at 3 to 12 d and 21 to 24 d after PS. The plasma FSH profiles followed a similar pattern in AL and RF birds (Figure 5-6), although the AL values were only significantly greater from 3 to 6 d after PS. A body size effect was observed from 12 to 15 d after PS, when FSH concentrations of the STD birds were greater than HIGH bird values.

Both plasma LH and FSH concentrations appeared to be at a basal level at PS (Figures 5-5 and 5-6). Following PS, both hormones typically increased to peak values within 3 d, and from this point gradually declined as sexual maturation proceeded, presumably in response to the negative feedback effects of increasing estradiol-17β concentrations on the hypothalamic stimulation of LH and FSH release. Vanmontfort *et al.* (1995), using White Leghorn chickens, reported similar LH values and lower FSH values to those of the current study. They also showed similar declines in LH concentration in time as the plasma steroid concentration rose. Prior to PS, however, when steroid concentrations were at baseline, they reported the LH concentration profile to proceed in a series of peaks and troughs. As their birds were maintained throughout rearing under photostimulation conditions (14L:10D), it is not known if the LH peaks prior to PS would also have occurred in birds of the current study. In a study by Williams and Harvey (1986), pullets were on a gradual PS program, but LH concentrations did not change dramatically during this period. However, strain differences have been reported for LH concentrations in poultry (Scanes *et al.*, 1980), with higher plasma LH concentrations in broiler type birds than in egg laying strains.

The significantly greater plasma concentrations of both LH and FSH in AL birds compared to RF birds within 3 d of photostimulation (Figures 5-5 and 5-6) provide evidence for a link between nutrition and reproduction. In primates, normal changes in the body's metabolism during the transition from juvenile to mature adult are thought to influence the rate of maturation of the neuroendocrine system and therefore the timing of puberty (Steiner, 1987). Blood-borne metabolic factors, glucose, amino acids, and insulin in particular, were found to augment LH secretion in immature animals. As basal levels of factors such as insulin differed between immature and adult animals (Steiner, 1987), energy balance of the animal may also be important in the sexual maturation process. This may partly explain the hormonal differences observed following PS between AL and RF birds in the current experiment, as well as the time difference in reaching SM. The difference in LH and FSH concentrations between the feeding regimens is purely a feed effect, as birds from these two feeding regimens originated from the same body size groups and body size did not initially affect the concentrations of these hormones. Feed restriction in egg-type hens is known to reduce LH concentrations (Tanabe *et al.*, 1981) and, in mammals, this occurs through altered LHRH secretion (Steiner, 1987; Cosgrove *et al.*, 1991).

The initial FSH concentration for the first sample following PS was exceptionally high in a number of birds. It is not known if this "spike" is characteristic of these broiler breeders following PS, as the 3 d sampling interval did not provide adequate resolution to precisely characterize the initial hormone responses. Biologically active LH and FSH is stored in secretory vesicles just under the plasma membrane of the secretory cells. Hormone signaling causes immediate exocytosis of the peptide hormone into the blood and stimulates synthesis of replacement hormone stores (Darnell *et al.*, 1986). The secretory cells typically have one to several days supply of hormone stored, which coincides with the transient peak LH and FSH concentrations observed in the birds of the current study.

The estradiol-17β profile of the AL birds spans a similar range of concentrations to the RF birds (Figure 5-4), resulting in a similar mean estradiol-17β concentration despite the presence of significantly greater AL concentrations between 18 to 21 and 27 to 30 d after PS compared to RF bird values. Plasma estradiol-17β concentration in the turkey will typically increase within 4 d of PS, peak just prior to SM, and then drop by about 30% to a level maintained (excluding age effects) for the duration of the laying period (Bacon *et al.*, 1980). Examination of the estradiol-17β profiles within the Feed X Size interaction shows this to be true in the current experiment as well, although there appears to be a delay in the hormone increase of the RF-LOW birds (Figure 5-4). The rate of change in plasma estradiol-17β concentration between PS and peak estradiol-17β concentration was reduced in the LOW birds compared to the HIGH birds (5.81 *vs.* 9.78 pg/d for LOW and HIGH birds, respectively) (Table 5-12), indicating a slowed establishment of increased estradiol-17β concentrations in LOW body size birds. Possibly as a result of extreme treatment and bird variation in the timing of the peak estradiol-17β concentration and SM, the Kolmogorov-Smirnoff analysis of the profiles revealed no differences in their shape.

The day of peak estradiol-17β concentration occurred between 5.69 d (RF birds) and 6.63 d (AL birds) prior to SM and was very consistent within each feeding regimen (Table 5-12). Alignment of the plasma estradiol-17β profiles for each bird with the physiological event of peak estradiol-17β concentration produced similar curves for the main treatment effects and their interaction (Figure 5-7). Kolmogorov-Smirnoff analysis of the profiles in this form showed them to be similar. Examination of the AL and RF profile clearly demonstrates the extended resting period that existed in the RF birds prior to the formation of an estradiol-17β profile similar in shape to that of the AL birds beginning at about 30 d prior to peak estradiol-17β concentration. This resting period is particularly apparent in the RF-LOW and RF-STD groups within the Feed X Size interaction.

The peak plasma estradiol-17β concentration of RF birds was greater than that of the AL birds (236.1 *vs.* 185.3 pg/mL for RF and AL birds, respectively). (Table 5-12) (Figure 5-7). At this time plasma LH and FSH concentrations were greater in AL than RF birds. Although this may relate to reduced levels of negative steroid feedback in AL birds, the concentrations of both LH and FSH were greater in AL than in RF bird plasma for up to 15 d prior to peak estradiol-17β concentration (Figures 5-8 and 5-9) and more likely represent the differences due to nutritional status between these groups.

The significantly reduced peak estradiol-17β concentration in AL birds and numerically reduced values for most other points relative to RF values has also been observed in growth selected turkey stocks compared to reproductively selected stocks (Melnychuk *et al.*, 1997). Fatty tissue in humans has been reported to convert estradiol to estriol (Fishman *et al.*, 1975), which can possibly either reduce target tissue response or enhance stimulation through a more biologically active estrogen form. The concentration of the plasma steroid carrier, sex hormone binding globulin (SHBG), is inversely related to insulin concentration in a BW and fat content-dependent manner (Botwood *et al.*, 1995) and is reported to be stimulated by estrogen treatment (Toscano *et al.*, 1992). In the current study, the plasma lipid concentrations in AL birds were 84% higher than those of RF birds (Table 5-12), and AL birds were fatter (Tables 5-6 and 5-7).

increased feed intake in AL compared to RF birds (Table 5-4) may also affect plasma estradiol-17β concentrations through steroid clearance and recycling mechanisms. Oats, barley chaff, and wheat chaff are excellent binders of estradiol-17β in the gut (Arts *et al.*, 1991), and are utilized in human diets to reduce estrogen exposure. These estrogen-binding feed components are all present in some form in the mash diets fed in the current study (Table 5-1). The reduced rate of passage in the gut associated with restricted feeding in sheep has been implicated for an increased overall estrogenic activity in plasma due to reduced steroid clearance rates and possibly to improved hormone resorption (Adams *et al.*, 1994).

Yolk size in RF birds was related to peak plasma estradiol-17β concentration. Peak estradiol-17β concentration correlated with the total weight of the LYF ($r=0.512$; $P=0.005$) and with weights of the individual LYF smaller than the F3 follicle (third largest LYF) (P range of =0.015 to =0.032 for F4 to F8). In a study of the effects of estradiol-17β implants in 59 wk old broiler breeders, Renema *et al.* (1997c) found that the estradiol-17β implants increased ovary weight by an average of 9% over blank implanted bird values due to an increase in mean LYF weight. These data suggest that increased estradiol-17β concentrations stimulate increased rates of yolk synthesis, which results in a larger egg yolk size.

It has previously been shown that birds considerably smaller than the mean BW at PS produce fewer eggs than birds at the target BW or high BW birds (Robinson and Robinson, 1991). This conclusion is supported by the current study, where the LOW birds reached SM much later than STD and HIGH birds, thereby hindering potential egg production. Although relative carcass traits were comparable between the size classes, the number of LYF in the RF-LOW birds was numerically reduced at SM and the number of small atretic follicles significantly increased. As LYF numbers will continue to decline with age, this combination of slightly lower LYF number with increased rates of follicular atresia is likely detrimental to potential egg production. The use of an AL feeding regimen with these birds significantly accelerates the sexual maturation process and increases carcass fatness, plasma lipid concentrations, and ovarian LYF numbers. The inferior LYF arrangement on the AL bird ovaries, as indicated by the high proportion of follicles in a multiple hierarchical arrangement compared to RF bird ovaries (Table 5-10), concurs with previous studies with overfed birds (Hocking *et al.*, 1987, 1989; Katanbaf *et al.*, 1989; Yu *et al.*, 1992a) and indicates an increased potential for production of unsettable eggs. The AL feeding regimen stimulated increased LH and FSH production compared with concentrations recorded in RF birds, suggesting that energy balance may interact with or modulate BW and age threshold effects in the initiation of sexual maturation. However, plasma LH and FSH concentrations in RF birds remained elevated for a longer time period than in AL birds. The extended time period for which the ovary was exposed to high LH and FSH levels may be essential for the normal development of mechanisms controlling LYF numbers, such as the mechanism of small follicle atresia.

Table 5-1. Composition and analysis of experimental diets

Ingredient and analysis	Starter (0 to 3 wk)	Grower (3 to 21 wk)	Breeder (21 wk to SM)
		(%)	
Ground wheat	44.23	34.42	33.76
Ground corn	14.18	16.44	14.31
Ground oats	5.00	12.50	10.00
Soybean meal (48%C.P.)	17.34	7.37	13.42
Ground barley	5.00	10.00	15.00
Wheat shorts	7.50	15.00	1.29
Limestone	1.65	1.72	7.68
Dicalcium phosphate	1.58	0.86	1.06
Choline chloride premix[1]	0.50	0.50	0.50
Broiler microingredient premix[2]	0.50	0.50	
Layer microingredient premix[3]			0.50
Iodized salt	0.35	0.33	0.28
L-lysine HCL	0.03	0.16	0.03
DL methionine	0.14	0.13	0.17
Tallow	2.00	0.07	2.00
Rumensin	0.08	0.05	0.00
Calculated Analysis			
ME (Kcal/kg)	2875	2706	2750
CP (%)	15.49	14.98	15.49
Calcium (%)	1.00	0.90	3.19
Available P (%)	0.70	0.56	0.42
Lysine (%)	0.90	0.75	0.75
Methionine (%)	0.41	0.35	0.41

[1] Provided choline chloride in the diet at a level of 100 mg/kg.

[2] Broiler premix provided per kilogram of diet: vitamin A (retinyl acetate), 10,000 IU; cholcalciferol, 2,500 IU; vitamin E (DL-α-tocopheryl acetate), 35 IU; vitamin K, 2.0 mg; pantothenic acid, 14 mg; riboflavin, 5.0 mg; folacin, 0.8 mg; niacin, 65 mg; thiamine, 2.0 mg; pyridoxine, 4.0 mg; vitamin B12, 0.015 mg; biotin, 0.18 mg; iodine, 0.5 mg; Mn, 70 mg; Cu, 8.5 mg; Zn, 80 mg, Se, 0.1 mg; Fe, 100 mg.

[3] Layer premix provided per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU; cholcalciferol, 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 40 IU; vitamin K, 2.0 mg; pantothenic acid, 14 mg; riboflavin, 6.5 mg; folacin, 1.0 mg; niacin, 40 mg; thiamine, 3.3 mg; pyridoxine, 6.0 mg; vitamin B12, 0.02 mg; biotin, 0.2 mg; iodine, 0.5 mg; Mn, 75 mg; Cu, 15 mg; Zn, 80 mg, Se, 0.1 mg; Fe, 100 mg.

Table 5-2. Total feed allowances for birds on the FF or SF feeding regimen between 20 wk of age and sexual maturity (first oviposition)

Age (wk)	Restricted feeding regimen[1] LOW	STD	HIGH
	(g per bird per d)		
21 to 22	98	100	104
22 to 23[2]	98	105	111
23 to 24	98	108	116
24 to 25	100	111	120
25 to 26	102	115	123
26 to 27	105	121	128
27 to 28	109	126	130
28 to 29	111	128	–
29 to 30	111	128	--

[1] STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively, than STD birds. STD birds fed to match BW targets. LOW and HIGH birds fed to maintain similar rates of gain to STD birds.

[2] Feed increases greater than 3 g split into 2 to 3 smaller increases throughout the week.

Table 5-3. Carcass characteristics and carcass composition traits of LOW, STD, and HIGH BW birds at photostimulation (21 wk of age)

Parameter	Body size[1]			SEM
	LOW	STD	HIGH	
	(Mean)			
BW (g)	1639^{c}	1995^{b}	2394^{a}	27
Shank length (mm)	99.9^{b}	103.1^{a}	105.3^{a}	0.9
Breast muscle weight				
(g)	234.0^{c}	327.0^{b}	395.8^{a}	7.1
(% of BW)	14.25^{b}	16.39^{a}	16.54^{a}	0.39
Liver weight				
(g)	38.1^{b}	32.0^{c}	52.1^{a}	2.1
(% of BW)	2.32^{a}	1.60^{b}	2.17^{a}	0.09
Abdominal fatpad weight				
(g)	8.7^{c}	19.4^{b}	31.6^{a}	3.0
(% of BW)	0.53^{b}	0.96^{a}	1.32^{a}	0.14
Oviduct weight				
(g)	0.44^{b}	1.00^{ab}	1.21^{a}	0.19
(% of BW)	0.027	0.050	0.051	0.008
Ovary weight				
(g)	0.59^{b}	0.93^{a}	1.00^{a}	0.08
(% of BW)	0.036	0.047	0.042	0.004
Carcass Composition:				
Protein				
(g)	328.7^{c}	419.4^{b}	487.1^{a}	7.8
(% of BW)	20.0	21.0	20.3	0.3
Lipid				
(g)	102.9^{c}	179.5^{b}	240.9^{a}	12.2
(% of BW)	6.3^{b}	8.9^{a}	10.1^{a}	0.6
Ash				
(g)	56.7^{c}	66.4^{b}	79.8^{a}	1.7
(% of BW)	3.4	3.3	3.3	0.1
Water				
(g)	1146^{c}	1321^{b}	1584^{a}	18
(% of BW)	70.0^{a}	66.3^{b}	66.2^{b}	0.6

$^{a-b}$Means within a row with no common superscript differ significantly ($P<0.05$).

[1] STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

Table 5-4. Body weight, rate of gain, and feed intake in standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity

			BW gain			Feed intake		
Source	BW at PS[1]	BW at SM	Total gain PS to SM	Daily gain PS to 23 wk	Daily gain PS to SM	Total feed PS to SM	Daily feed PS to 23 wk	Daily feed PS to SM
	(g)	(g)	(g)	(g/d)	(g/d)	(g)	(g/d)	(g/d)
Feed[3]								
AL	1946	3599^{a}	1653^{a}	83.9^{a}	66.6^{a}	6486^{a}	223.5^{a}	255.1^{a}
RF	1962	2864^{b}	902^{b}	18.7^{b}	23.7^{b}	4163^{b}	102.6^{b}	106.8^{b}
SEM	18	42	46	1.9	1.2	269	2.2	2.7
Size[4]								
LOW	1574^{c}	3075^{b}	1501^{a}	53.7^{a}	43.7	6340^{a}	153.8^{b}	177.7
STD	1948^{b}	3243^{a}	1295^{b}	54.6^{a}	45.8	5434^{b}	168.7^{a}	185.9
HIGH	2340^{a}	3377^{a}	1037^{c}	45.6^{b}	46.0	4199^{b}	166.0^{a}	179.3
SEM	22	52	56	2.3	1.5	332	2.5	3.4
Interaction								
AL-LOW	1566^{c}	3397^{b}	1831^{a}	85.2^{a}	64.1	7422^{a}	209.6^{b}	253.5^{ab}
AL-STD	1932^{b}	3627^{ab}	1695^{a}	90.9^{a}	68.0	6626^{ab}	234.9^{a}	262.8^{a}
AL-HIGH	2341^{a}	3773^{a}	1432^{b}	75.5^{b}	67.6	5409^{b}	225.9^{a}	248.9^{b}
RF-LOW	1582^{c}	2752^{b}	1171^{a}	22.1	23.2	5259^{a}	98.0	101.8
RF-STD	1964^{b}	2858^{ab}	894^{b}	18.4	23.6	4241^{ab}	102.5	109.1
RF-HIGH	2339^{a}	2981^{a}	641^{c}	15.6	24.4	2989^{b}	107.5	109.7
SEM	32	76	82	3.3	2.1	482	3.9	4.6
	Probability							
Source of variation								
Feed	0.54	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Size	0.0001	0.0005	0.0001	0.011	0.46	0.0001	0.0005	0.18
Feed X Size	0.86	0.56	0.61	0.13	0.69	0.95	0.026	0.24

$^{a-c}$Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Body weight at photostimulation.

[2] Body weight at sexual maturity (first oviposition).

[3] AL = *ad libitum* fed; RF = restricted fed.

[4] STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

Table 5-5. Time of sexual maturity and first egg weight of standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity

Source	Days from PS to SM[1]	First egg weight
	(d)	(g)
Feed[2]		
AL	25.3^{b}	48.6
RF	38.9^{a}	46.7
SEM	1.7	1.0
Size[3]		
LOW	40.3^{a}	50.1^{a}
STD	31.6^{b}	46.6^{b}
HIGH	24.2^{c}	46.2^{b}
SEM	2.1	1.2
Interaction		
AL-LOW	29.1	50.3
AL-STD	25.1	47.7
AL-HIGH	21.5	47.9
RF-LOW	51.4^{a}	50.0^{a}
RF-STD	38.4^{b}	45.5^{ab}
RF-HIGH	26.9^{c}	44.6^{b}
SEM	3.1	1.7
	Probability	
Source of variation		
Feed	0.0001	0.17
Size	0.0001	0.047
Feed X Size	0.025	0.68

[a-c] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Days from photostimulation to sexual maturity (first oviposition).

[2] AL = *ad libitum* fed; RF = restricted fed.

[3] STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

Table 5-6. Breast muscle, abdominal fatpad, and liver measurements at processing in standard, low, and high BW broiler breeders at sexual maturity following *ad libitum* or restricted feeding from photostimulation (21 wk of age)

	Breast muscle		Abdominal fatpad		Liver		Liver lipid	
Source	Weight	Percentage^{1}	Weight	Percentage	Weight	Percentage	Weight	content^{2}
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Feed^{3}								
AL	489.7^{a}	14.59^{b}	124.0^{a}	3.68^{a}	78.9^{a}	2.34^{a}	16.1^{a}	18.00^{a}
RF	446.7^{b}	16.20^{a}	55.0^{b}	1.96^{b}	42.4^{b}	1.54^{b}	3.0^{b}	6.62^{b}
SEM	7.7	0.23	4.4	0.13	3.1	0.09	1.7	1.42
Size^{4}								
LOW	442.2^{b}	15.47	83.9	2.82	53.7^{b}	1.84	6.2	9.15^{b}
STD	469.6^{a}	15.13	86.9	2.68	63.9^{ab}	2.00	10.8	12.76^{ab}
HIGH	492.8^{a}	15.59	97.7	2.96	64.4^{a}	1.98	11.7	15.02^{a}
SEM	9.5	0.28	5.4	0.16	3.8	0.11	2.2	1.76
Interaction								
AL-LOW	461.9^{b}	14.87	116.5	3.73	68.6^{b}	2.19	10.3^{b}	12.92^{b}
AL-STD	490.2^{ab}	14.27	120.2	3.50	82.1^{ab}	2.38	18.4^{ab}	19.03^{ab}
AL-HIGH	517.0^{a}	14.65	135.4	3.83	86.0^{a}	2.46	19.6^{a}	22.06^{a}
RF-LOW	422.5^{b}	16.06	51.3	1.91	38.9	1.48	2.1	5.38
RF-STD	449.1^{ab}	16.00	53.7	1.86	45.7	1.63	3.1	6.50
RF-HIGH	468.6^{a}	16.53	60.0	2.10	42.7	1.51	3.7	7.80
SEM	13.7	0.41	7.8	0.23	5.5	0.17	3.1	2.55
	Probability							
Source of variation								
Feed	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Size	0.002	0.48	0.16	0.44	0.088	0.52	0.16	0.064
Feed X Size	0.94	0.66	0.76	0.91	0.44	0.72	0.37	0.39

[a-c] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Percentage = tissue weight / BW X 100.

[2] Lipid percentage = liver lipid weight / liver weight X 100.

[3] AL = *ad libitum* fed; RF = restricted fed.

[4] STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

Table 5-7. Carcass protein, lipid, ash and water content at processing in standard, low, and high BW broiler breeders at sexual maturity following *ad libitum* or restricted feeding from photostimulation (21 wk of age)

	Carcass protein		Carcass lipid		Carcass ash		Carcass water	
Source	Weight	Percentage[1]	Weight	Percentage	Weight	Percentage	Weight	Percentage
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Feed[2]								
AL	667.8^a	18.55^b	740.1^a	20.49^a	103.6^a	2.88^b	2084^a	57.99^b
RF	595.0^b	20.78^a	370.1^b	12.77^b	96.3^b	3.37^a	1801^b	63.02^a
SEM	9.4	0.18	19.2	0.43	1.7	0.05	23	0.46
Size[3]								
LOW	590.4^c	19.36^b	525.6	16.54	92.8^c	3.05	1864^b	60.99^a
STD	628.2^b	19.51^b	539.4	16.08	100.1^b	3.12	1976^a	61.32^a
HIGH	675.5^a	20.12^a	600.4	17.26	107.0^a	3.20	1988^a	59.22^b
SEM	11.7	0.21	23.7	0.53	2.1	0.06	28	0.56
Interaction								
AL-LOW	611.5^c	18.02^b	699.5	20.49	95.73^b	2.83	1987^b	58.56
AL-STD	669.8^b	18.46^{ab}	729.2	20.07	105.5^a	2.91	2124^a	58.60
AL-HIGH	721.9^a	19.16^a	791.7	20.91	109.6^a	2.91	2142^a	56.83
RF-LOW	569.3^b	20.70	351.8	12.60	89.9^b	3.27^b	1741	63.41^{ab}
RF-STD	586.6^{ab}	20.56	349.6	12.09	94.7^b	3.34^{ab}	1828	64.05^a
RF-HIGH	629.0^a	21.09	409.0	13.62	104.4^a	3.50^a	1833	61.60^b
SEM	16.9	0.32	34.4	0.77	3.0	0.08	41	0.83
	Probability							
Source of variation								
Feed	0.0001	0.0001	0.0001	0.0001	0.004	0.0001	0.0001	0.0001
Size	0.0001	0.035	0.19	0.28	0.0001	0.16	0.004	0.021
Feed X Size	0.27	0.44	0.50	0.88	0.57	0.50	0.70	0.89

[a-c] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Percentage = component weight / BW X 100.

[2] AL = *ad libitum* fed; RF = restricted fed.

[3] STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

Table 5-8. Oviduct, ovary, and stroma weights at processing in standard, low, and high BW broiler breeders at sexual maturity following *ad libitum* or restricted feeding from photostimulation (21 wk of age)

	Oviduct		Ovary		Initial ovarian stroma[3]		Bare ovarian stroma[4]	
Source	Weight	Percentage[2]	Weight	Percentage	Weight	Percentage	Weight	Percentage
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Feed[5]								
AL	62.4	1.86^{b}	82.1^{a}	2.45^{a}	8.63	0.257	3.59^{a}	0.106
RF	59.2	2.15^{a}	59.4^{b}	2.16^{b}	7.54	0.274	2.70^{b}	0.098
SEM	1.6	0.04	2.8	0.08	0.42	0.013	0.18	0.005
Size[6]								
LOW	61.8	2.16^{a}	70.6	2.42	8.58	0.296	3.26	0.112
STD	59.5	1.91^{b}	72.3	2.31	7.91	0.255	3.08	0.099
HIGH	61.0	1.93^{b}	69.5	2.18	7.78	0.244	3.09	0.095
SEM	1.9	0.05	3.4	0.10	0.52	0.016	0.22	0.007
Interaction								
AL-LOW	61.0	1.95	83.8	2.65	8.76	0.279	3.44	0.109
AL-STD	61.6	1.79	82.2	2.41	8.48	0.249	3.40	0.099
AL-HIGH	64.4	1.83	80.2	2.28	8.66	0.244	3.93	0.110
RF-LOW	62.6	2.38^{a}	57.5	2.19	8.40	0.314	3.09	0.115
RF-STD	57.4	2.03^{b}	62.4	2.22	7.33	0.262	2.76	0.098
RF-HIGH	57.6	2.03^{b}	58.8	2.07	6,90	0.245	2.26	0.080
SEM	2.8	0.07	4.9	0.15	0.75	0.022	0.31	0.009
	Probability							
Source of variation								
Feed	0.16	0.0001	0.0001	0.017	0.069	0.35	0.0008	0.25
Size	0.69	0.002	0.84	0.23	0.50	0.054	0.79	0.16
Feed X Size	0.30	0.24	0.78	0.57	0.63	0.73	0.092	0.14

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Days from photostimulation to sexual maturity (first oviposition).

[2] Percentage = tissue weight / BW X 100.

[3] Initial stroma = ovary without the large yellow follicles (follicles >10mm diameter removed).

[4] Bare stroma = initial stroma without the small yellow follicles or large white follicles (follicles >1mm diameter removed).

[5] AL = *ad libitum* fed; RF = restricted fed.

[6] STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

Table 5-9. Ovarian follicle numbers at processing in standard, low, and high BW broiler breeders at sexual maturity following *ad libitum* or restricted feeding from photostimulation (21 wk of age)

	Ovarian Follicles[1]			
Source	LYF	SYF	LWF	MWF
	(#)			
Feed[6]				
AL	11.0[a]	12.6	17.7	186.5
RF	7.1[b]	11.7	16.6	161.0
SEM	0.3	1.2	1.3	9.9
Size[7]				
LOW	8.8	13.3	18.4	174.6
STD	9.5	10.8	17.8	176.1
HIGH	8.9	12.4	15.3	170.5
SEM	0.4	1.5	1.6	12.2
Interaction				
AL-LOW	11.2	14.0	17.6	194.4
AL-STD	11.4	11.4	20.7	203.9
AL-HIGH	10.3	12.4	14.8	161.3
RF-LOW	6.3	12.7	19.1	154.8
RF-STD	7.6	10.1	14.9	148.2
RF-HIGH	7.4	12.4	15.8	179.7
SEM	0.6	2.1	2.4	17.7
	Probability			
Source of variation				
Feed	0.0001	0.60	0.56	0.070
Size	0.37	0.44	0.36	0.94
Feed X Size	0.23	0.93	0.21	0.078

[a,b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] LYF = large yellow follicles (>10mm diameter); SYF = small yellow follicles (5-10mm diameter); LWF = large white follicles (3-5mm diameter); MWF = medium white follicles (1-3mm diameter).

Table 5-10. Ovarian small follicle atresia, unexplained POF, and LYF hierarchy weight and arrangement parameters at processing in standard, low, and high BW broiler breeders at sexual maturity following *ad libitum* or restricted feeding from photostimulation (21 wk of age)

			LYF parameters					
Source	Small atretic follicles[1]	Unexplained POF[2]	F1 weight	Mean weight	Number in multiple sets[3]	Percentage in multiple sets[3]	Hierarchy positions[4]	Number of hierarchies[5]
	(#)	(g)	(g)	(g)	(#)	(%)	(#)	(#)
Feed[6]								
AL	10.3^{b}	2.13^{a}	12.12	6.71^{b}	8.46^{a}	74.2^{a}	6.50^{a}	1.70^{a}
RF	32.3^{a}	0.31^{b}	12.59	7.39^{a}	2.59^{b}	33.7^{b}	5.79^{b}	1.23^{b}
SEM	2.7	0.17	0.22	0.17	0.48	4.1	0.16	0.05
Size[7]								
LOW	26.5	0.86	13.07^{a}	7.23	5.53	52.8	5.98	1.46
STD	20.6	1.40	11.99^{b}	6.87	6.25	61.0	6.35	1.49
HIGH	16.8	1.41	12.00^{b}	7.04	4.80	48.2	6.10	1.44
SEM	3.3	0.21	0.27	0.21	0.60	5.1	0.20	0.06
Interaction								
AL-LOW	10.7	1.50^{b}	12.40	6.62	9.50	81.8	6.30^{ab}	1.79
AL-STD	10.6	2.30^{ab}	11.78	6.46	8.90	77.3	7.00^{a}	1.65
AL-HIGH	9.5	2.60^{a}	12.17	7.03	7.00	63.7	6.20^{b}	1.65
RF-LOW	42.2^{a}	0.22	13.74^{a}	7.83	1.56	23.8	5.67	1.23
RF-STD	30.6^{ab}	0.22	12.20^{ab}	7.28	3.60	44.7	5.70	1.34
RF-HIGH	24.0^{b}	0.50	11.82^{b}	7.06	2.60	32.7	6.00	1.24
SEM	4.9	0.28	0.39	0.31	0.86	7.4	0.29	0.08
	Probability							
Source of variation								
Feed	0.0001	0.0001	0.13	0.007	0.0001	0.0001	0.003	0.0001
Size	0.12	0.11	0.007	0.49	0.22	0.19	0.40	0.83
Feed X Size	0.19	0.18	0.091	0.13	0.097	0.11	0.14	0.072

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Number of atretic follicles < 5 mm in diameter.

[2] Post ovulatory follicles not accounted for by eggs laid, or by yolks or eggs in oviduct.

[3] Follicles arranged in groups differing by < 1 g.

[4] Number of groups of follicles within 1 g.

[5] Hierarchies calculated as LYF divided by positions.

[6] AL = *ad libitum* fed; RF = restricted fed.

[7] STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

Table 5-11. Photostimulation (PS), sexual maturity (SM) and mean plasma estradiol-17β (E2), luteinizing hormone (LH), and follicle stimulating hormone (FSH) concentration in standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between PS (21 wk of age) and SM

	PS hormone concentrations			SM hormone concentrations			Mean hormone concentrations[1]		
Source	E2	LH	FSH	E2	LH	FSH	E2	LH	FSH
	(pg/mL)	(ng/mL)	(ng/mL)	(pg/mL)	(ng/mL)	(ng/mL)	(pg/mL)	(ng/mL)	(ng/mL)
Feed[2]									
AL	40.7	2.65	1.36	104.5	3.49	1.64	113.4	4.40^{a}	3.47
RF	38.1	2.25	1.25	94.4	3.22	1.70	113.3	3.38^{b}	2.85
SEM	3.7	0.23	0.15	7.2	0.40	0.16	7.6	0.13	0.27
Size[3]									
LOW	33.4^{b}	2.26	1.20	97.5	2.96	1.56	97.8^{b}	3.69	3.21
STD	35.5^{b}	2.58	1.28	85.7	3.72	1.73	108.3^{b}	4.11	3.30
HIGH	49.1^{a}	2.52	1.43	115.1	3.38	1.73	134.1^{a}	3.87	2.98
SEM	4.6	0.28	0.18	8.9	0.33	0.19	9.2	0.16	0.33
Interaction									
AL-LOW	32.7^{b}	2.17	1.19	105.9^{ab}	3.36	1.57	109.4	4.26	3.71
AL-STD	36.8^{ab}	2.85	1.42	83.1^{b}	3.76	1.61	107.1	4.70	3.76
AL-HIGH	52.7^{a}	2.95	1.47	124.4^{a}	3.37	1.74	123.7	4.23	2.96
RF-LOW	34.1	2.35	1.22	89.0	2.56	1.55	86.2^{b}	3.13	2.71
RF-STD	34.3	2.31	1.15	88.3	3.69	1.85	109.4^{ab}	3.53	2.84
RF-HIGH	45.5	2.09	1.39	105.8	3.40	1.72	144.4^{a}	3.50	3.00
SEM	6.7	0.41	0.27	12.9	0.48	0.27	13.4	0.23	0.48
					Probability				
Source of variation									
Feed	0.58	0.21	0.62	0.32	0.47	0.77	0.99	0.0001	0.11
Size	0.037	0.70	0.67	0.062	0.28	0.77	0.022	0.19	0.79
Feed X Size	0.42	0.41	0.83	0.56	0.62	0.85	0.25	0.56	0.47

[a-b] Means within a column and within a source with no common superscript differ significantly. Interaction means are compared within a feed.

[1] Mean concentration of plasma hormone in samples taken at 3d intervals between PS and SM.

[2] AL = *ad libitum* fed; RF = restricted fed.

[3] STD = target BW birds; LOW and HIGH BW birds = naturally 20% lighter or heavier, respectively.

Table 5-12. Plasma lipid concentration, time of estradiol-17β (E2) concentration, plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations at peak E2, and E2 profile characteristics concentration in standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between PS (21 wk of age) and SM

Source	Plasma lipids at PS	Plasma lipids at SM	Days from PS to peak E2	Days from peak E2 to SM	Peak E2 hormone concentrations[1]			E2 difference		Rate of E2 change to peak
					E2	LH	FSH	PS to SM	PS to peak E2	
	(mg/mL)	(mg/mL)	(d)	(d)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/mL)	(pg/d)
Feed[2]										
AL	6.38	37.70^{a}	18.60^{b}	6.63	185.3^{b}	4.14^{a}	2.64^{a}	63.7	144.5^{b}	8.19
RF	6.48	20.54^{b}	33.78^{a}	5.69	236.1^{a}	3.61^{b}	1.94^{b}	56.4	196.9^{a}	7.12
SEM	0.14	2.28	1.63	0.48	13.8	0.17	0.23	8.0	13.7	0.78
Size[3]										
LOW	6.60	30.95	34.23^{a}	6.04	212.8	3.89	2.15	64.1	179.4	5.81^{b}
STD	6.26	24.26	25.50^{b}	6.25	197.7	3.98	2.20	50.2	162.1	7.38^{ab}
HIGH	6.43	30.66	18.83^{c}	6.19	221.5	3.74	2.51	66.0	170.6	9.78^{a}
SEM	0.17	2.82	1.97	0.58	16.7	0.20	0.28	9.9	16.7	0.95
Interaction										
AL-LOW	6.31	38.63	22.8	6.30	200.6	4.27	2.75	73.2	167.9	7.21
AL-STD	6.28	30.63	18.0	7.10	165.8	4.41	2.75	46.3	129.0	7.61
AL-HIGH	6.55	41.20	15.0	6.50	189.4	3.74	2.43	71.7	136.7	9.74
RF-LOW	6.89	23.36	45.7^{a}	5.78	225.1	3.52	1.55	54.9	191.0	4.40
RF-STD	6.24	18.25	33.0^{b}	5.40	229.6	3.56	1.65	54.0	195.2	7.15
RF-HIGH	6.32	20.12	22.7^{c}	5.89	253.6	3.74	2.62	60.4	204.5	9.82
SEM	0.25	4.09	2.86	0.85	24.3	0.29	0.41	14.3	24.3	1.38
	Probability									
Source of variation										
Feed	0.59	0.0001	0.0001	0.16	0.011	0.026	0.035	0.69	0.005	0.33
Size	0.38	0.17	0.0001	0.96	0.59	0.70	0.59	0.45	0.76	0.017
Feed X Size	0.23	0.51	0.031	0.72	0.63	0.27	0.15	0.32	0.45	0.52

[a-c] Means within a column and within a main effect or the interaction with no common superscript differ significantly.

[1] Concentration of E2, LH, and FSH at time of peak E2 concentration.

[2] Feeding regimens: FF = Fast feed; SF = Slow feed.

[3] Sexual maturity time: EARLY = first 50% of birds within each feeding regimen to reach SM; LATE = last 50% of birds within each feeding regimen to reach SM.

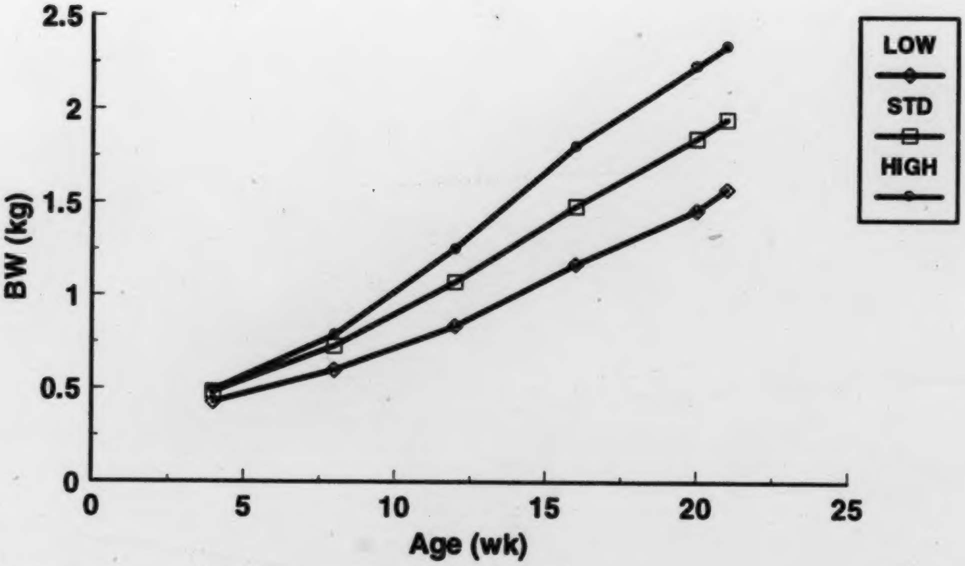

Figure 5-1. Body weights of standard (STD), low, and high BW pullets between 4 and 21 wk of age. The target BW curve is represented by STD birds, whereas the LOW and HIGH curves represent birds naturally 20% lighter or heavier, respectively.

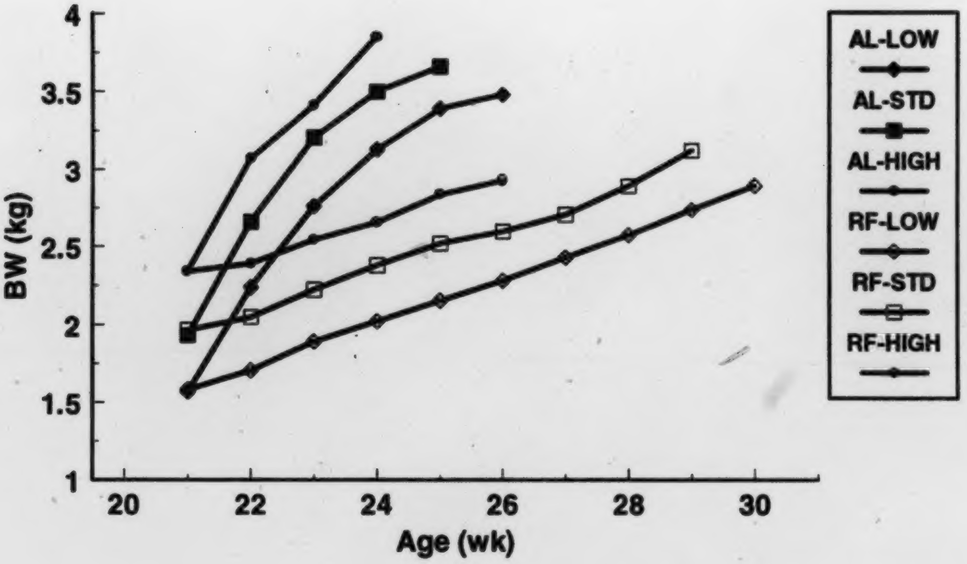

Figure 5-2. Body weights of standard (STD), low, and high BW broiler breeders either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity.

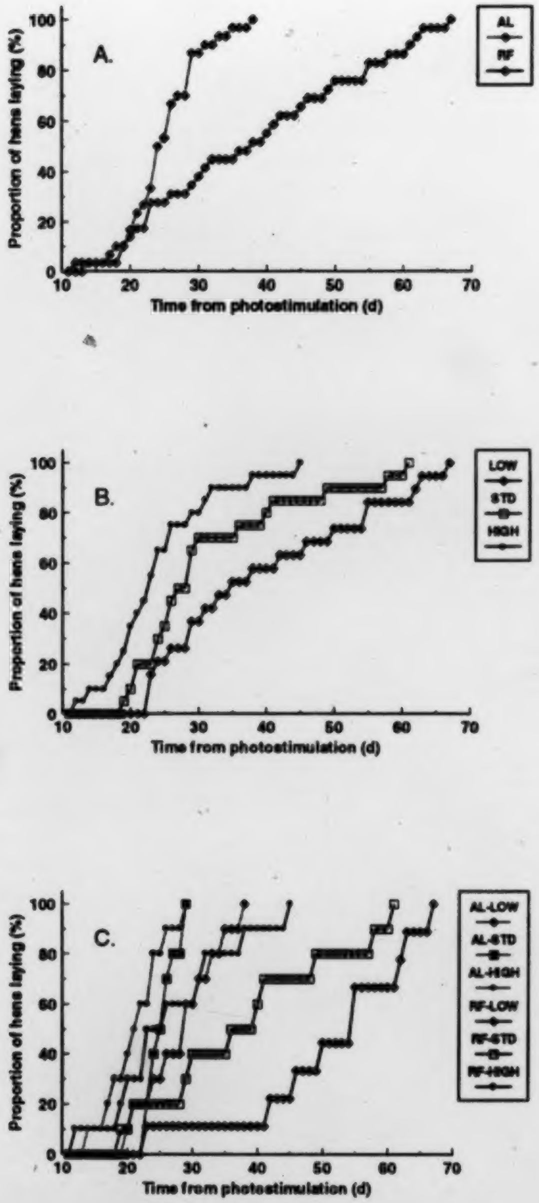

Figure 5-3. Additive curve of the proportion of broiler breeders reaching sexual maturity for the main effect, Feed (A), Size (B), and for the interaction of Feed X Size (C) in standard, low, and high BW birds either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity.

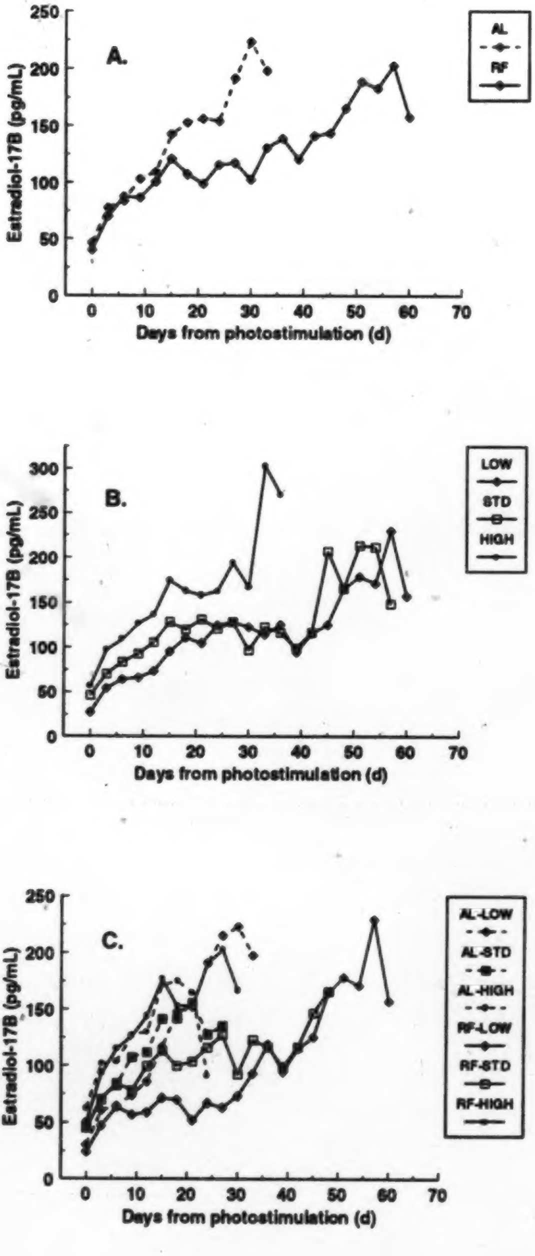

Figure 5-4. Plasma Estradiol-17β concentration profiles for the main effect, Feed (A), Size (B), and for the interaction of Feed X size (C) in standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity.

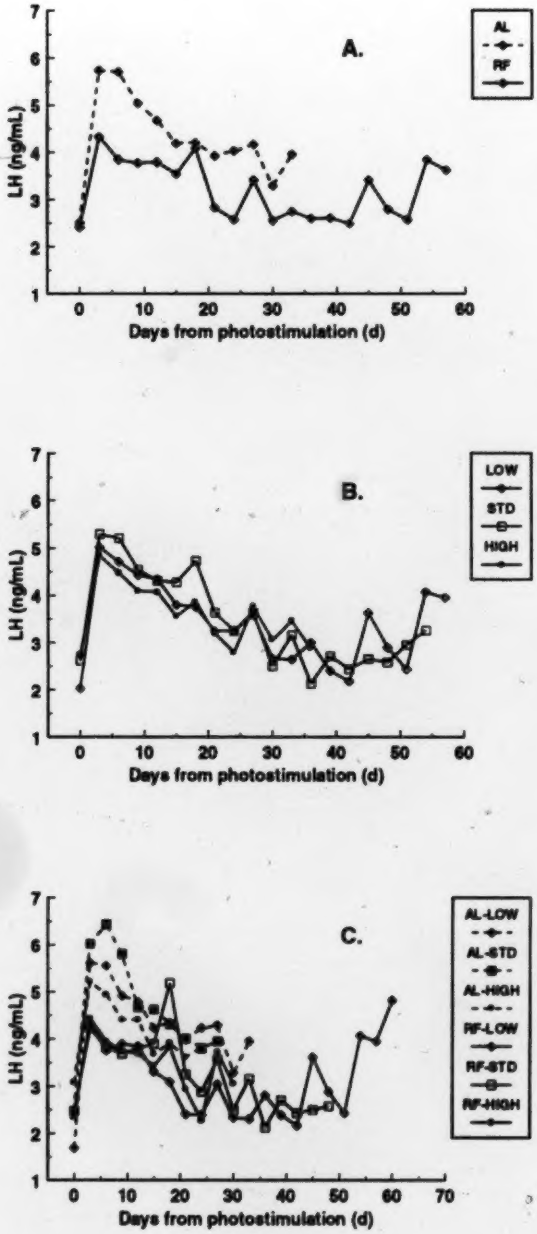

Figure 5-5. Plasma LH concentration profiles for the main effect, Feed (A), Size (B), and for the interaction of Feed X size (C) in standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity.

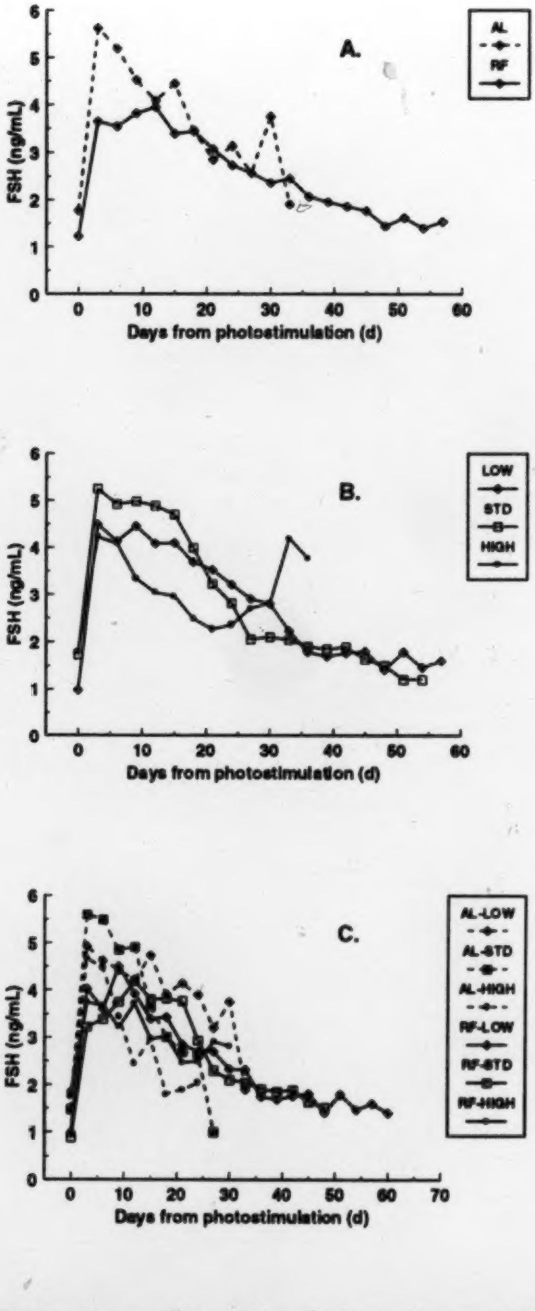

Figure 5-6. Plasma FSH concentration profiles for the main effect, Feed (A), Size (B), and for the interaction of Feed X size (C) in standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity.

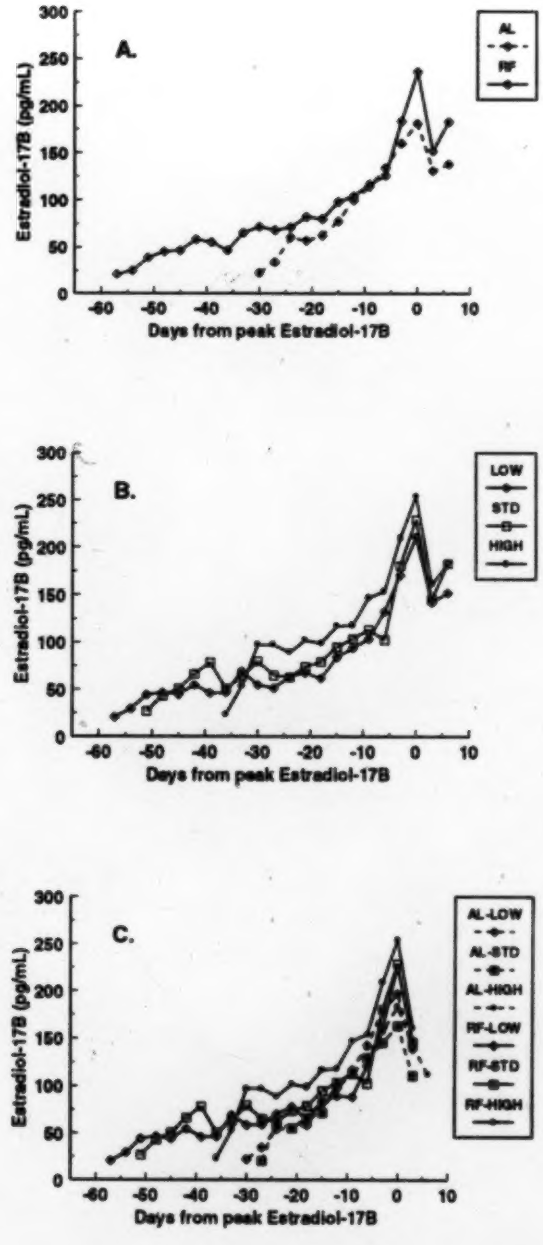

Figure 5-7. Plasma Estradiol-17β concentration profiles for the main effect, Feed (A), Size (B), and for the interaction of Feed X size (C) with the curves adjusted to day of peak Estradiol-17β as time = 0 in standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity.

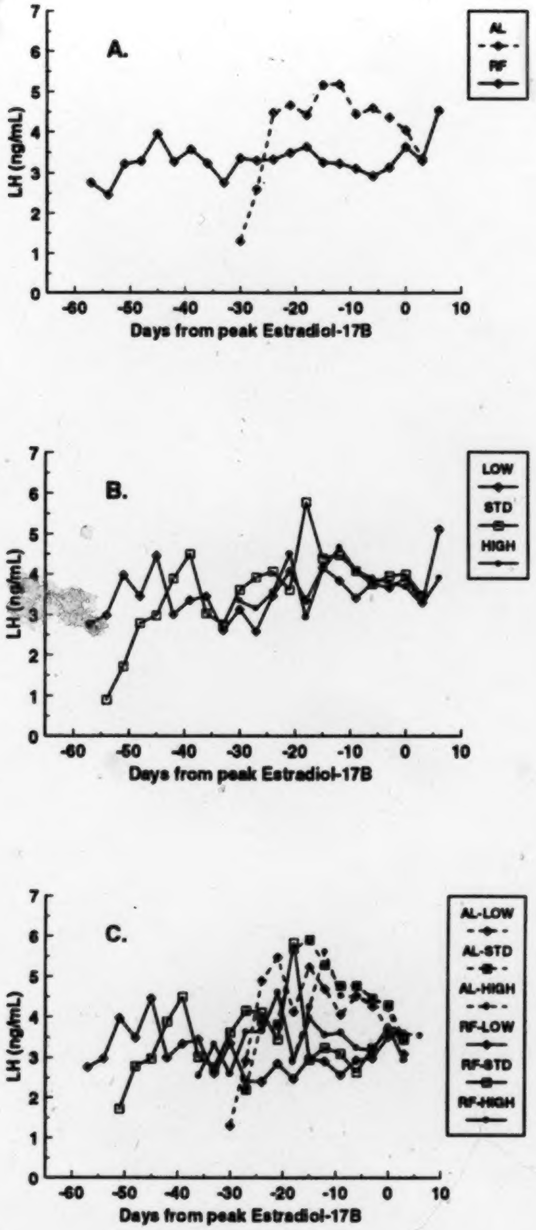

Figure 5-8. Plasma LH concentration profiles for the main effect, Feed (A), Size (B), and for the interaction of Feed X size (C) with the curves adjusted to day of peak Estradiol-17β as time = 0 in standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity.

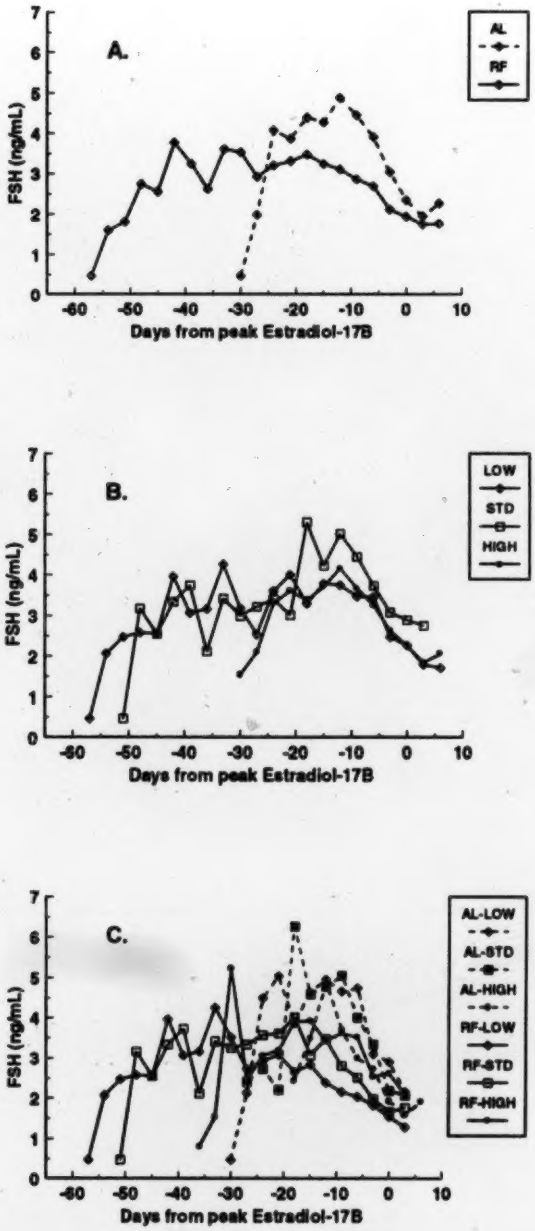

Figure 5-9. Plasma FSH concentration profiles for the main effect, Feed (A), Size (B), and for the interaction of Feed X size (C) with the curves adjusted to day of peak Estradiol-17β as time = 0 in standard, low, and high BW broiler breeders either *ad libitum* or restricted fed between photostimulation (21 wk of age) and sexual maturity.

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6. GENERAL DISCUSSION AND CONCLUSIONS

The effects of feeding level, age, and body size were examined in broiler breeder hens in an attempt to elucidate their effects on ovarian morphology and lipid allocation. Broiler breeder hen management in the 1990's is becoming a system of ovary management to maximize and maintain adequate egg production. The broiler breeder is a unique type of poultry because it has the genetics for rapid and efficient growth, but it needs to have good egg production to supply the next generation of broiler chicks. However, there is a negative relationship between BW and egg production in broiler breeders (Siegel and Dunnington, 1985), as there is in Japanese quail (Marks, 1985), and turkeys (Nestor *et al.*, 1980). Heavily growth-selected male-line turkey hens may be the most reproductively unfit hen in poultry production, with expected rates of lay being about half that of the reproductively-selected female-line hen (Hocking, 1992). Whereas broiler breeders lay better than turkeys, their expected egg production is half that of the very productive egg-type hens.

The level of follicular recruitment and the resulting number of ovarian large yellow follicles (LYF) is the source of the reproductive problems in broiler breeder stocks. Heavier broiler breeder strains have a greater number of LYF than lighter strains (Udale *et al.*, 1972; Reddy and Siegel, 1976). Body weight appears to be the primary effector of LYF number (Hocking and Whitehead, 1990; Hocking, 1993, 1996). Ovaries of growth-selected strains are sensitive to overfeeding during reproductive development. Extra feed at this time will result in the production of excess LYF which are likely to be arranged in multiple hierarchies (Hocking *et al.*, 1987, 1989; Katanbaf *et al.*, 1989; Yu *et al.*, 1992), thereby increasing numbers of unsettable eggs. Upon reviewing the ovary data from a number of broiler breeder trials, F. E. Robinson (personal communication) concluded that the ideal broiler breeder ovary has 7 to 8 LYF at sexual maturity (SM). Numbers greater than this result in the formation of multiple hierarchies.

In the current study, birds were examined at SM when excess LYF production is an issue, and later in lay when inadequate LYF numbers limit the rate of lay. Older hens have reduced rates of follicle recruitment and maturation (Yu *et al.*, 1992), as indicated by a reduced

LYF number and egg production. The ovaries of older birds contain fewer LYF which are larger in size than those of younger birds. The age-related decline in egg production could be due to reduced sensitivity of the hypothalamus to feedback from the steroid hormones (Williams and Sharp, 1978) and to increased rates of follicular atresia (Waddington *et al.*, 1985; Palmer and Bahr, 1992). The overfeeding of broiler breeders at 44 wk of age results in the formation of multiple hierarchies (Robinson *et al.*, 1993), but with fewer excess LYF than at SM. Although overfeeding broiler breeders results in a loss of regulation of the ovarian hierarchy in response to the extra feed, this response becomes more gradual in time. Hocking (1996) speculated that the sensitivity of the ovary to extra feed availability may be decreasing with broiler breeder age. By 59 wk of age, ovarian LYF numbers do not change with overfeeding (Renema, Thesis: Chapter 2), suggesting that the link between nutrition and reproduction is no longer inducible in this broiler breeder strain at this age.

Contrary to expectations, the use of a concentrated (CON) diet (5.3% more kcal and CP/kg than standard (STD) diet) between photostimulation (PS) and 40 wk of age was found not to affect egg production, although egg laying patterns were altered (Renema, Thesis: Chapter 4). Between 32 and 40 wk of age, sequence length was reduced and pause length increased in CON compared to STD birds. These differences are classic symptoms of overfeeding in broiler breeders and, based on previous experiments, if treatments had continued through to 65 wk of age a significant decrease in CON bird egg production would likely have been observed. This is further supported by the LYF arrangement in CON birds ovaries, where a greater proportion of LYF were in a multiple hierarchy arrangement despite a similar LYF number to STD birds. Robinson *et al.* (1993) stated that the uniformity of spacing of the LYF may be more important than the number in ensuring the single ovulation of follicles and hence the production of settable eggs.

The experimental high breast yield broiler breeder strain used in Chapter 4 was relatively insensitive to both the concentrated diet and to the estradiol-17β treatment. However, composition of the lipids of the plasma very low density lipoprotein (VLDL) fraction were found to relate to lipid allocation and ovarian morphology. A low plasma VLDL-triglyceride

(TG):phospholipid (PL) ratio was associated with an increased ovary and stroma weight, and a low relative abdominal fatpad weight relative to birds with a high TG:PL ratio. Both mean LYF weight and mean LWF weight were greater in low TG:PL ratio birds, and the incidence of small follicle atresia (<5mm diameter) was increased dramatically. Although VLDL composition was not related to egg production at this time, a low VLDL-TG:PL ratio may be correlated with an increased mean sequence length (P=0.07) and a longer prime sequence length (a characteristically long sequence in early lay) (P=0.06). These observations suggest that the ovaries of birds with a low VLDL-TG:PL ratio are in a more active state of follicular recruitment. These interactions occurred independently of bird BW, indicating that plasma lipid metabolism is at least partially an independent process.

Although increased rates of follicular atresia in the low TG:PL ratio birds may be the reason why LYF numbers do not differ from that of high TG:PL ratio birds, the emphasis on ovary maintenance and yolk deposition may have a positive effect on both egg (Bacon and Cherms, 1968) and presumably chick size (Shanaway, 1984). In this experiment (Renema, Thesis: Chapter 3) it appears that birds which do not maintain ovary weight are getting fatter. As flock feeding levels are based on either flock egg production rate or mean BW, birds allocating less energy to egg production will get fatter and birds allocating more energy to producing eggs may be overly restricted. The reproductive status of birds of this age (42 wk) may not be very uniform. Although birds may be the same size, some may be undergoing an age-related decline in reproductive output at a faster rate than others. This variability may be the reason why the middle-aged birds in the experiment of Chapter 4 were the only ones in which significant correlations between VLDL-TG:PL ratio and ovarian morphology traits were observed.

A reduced plasma VLDL-TG:PL ratio means that the bird is likely producing smaller VLDL particles than their high TG:PL ratio counterparts. Estrogen is known to stimulate liver production of VLDL of a smaller diameter (Griffin *et al.*, 1982) which is preferentially taken up by the ovarian follicles (Schneider *et al.*, 1990). An increased ovary or mean LYF weight would be expected to be associated with a low VLDL-TG:PL ratio as a result of the production of an increased proportion of smaller VLDL particles containing less TG (Chapman, 1980; Bacon *et al.*, 1982). The use of estradiol-17β implants in breeder hens at 59 wk of age resulted in a larger ovary weight than in birds receiving a blank implant due to increased mean LYF weight (Renema, Thesis: Chapter 2). In these birds there was a trend toward a lower TG content in the VLDL lipids (P=0.07), which suggests the presence of smaller VLDL particles. The weight of liver lipids was correlated with ovary weight in birds at 42 wk of age (Renema, Thesis: Chapter 4). This important link between the site of yolk lipid synthesis and deposition was not significantly affected by BW.

In the estradiol-17β implant studies (Renema, Thesis: Chapters 2 and 4), birds responded variably to the combination of feed and estrogen treatment. In Chapter 2, the best egg layers in the experimental group had the largest increases in plasma lipid concentrations because of a feed or estradiol-17β challenge, due in particular to an increased VLDL-TG content. The poor egg layers, with presumably higher VLDL-TG:PL ratios, may have been less able to alter their VLDL composition, as it was already in a form favoring allocation to sites or uses other than the ovary. In Chapter 4, estradiol-17β implants in birds on the CON feeding regimen were numerically less effective at increasing plasma estradiol-17β concentration than in the experiment of Chapter 2. The enriched nutrient environment of the CON bird plasma may have affected its steroid carrying potential, as the concentration of sex hormone binding globulin (SHBG) has been reported to be inversely related to plasma insulin concentration in a BW and carcass fat content-dependent manner (Botwood *et al.*, 1995).

The state of the ovary at SM has been reported to relate to the future reproductive performance of the hen, with differences of as little as one extra LYF resulting in a 10 egg reduction in subsequent egg production (Robinson *et al.*, 1995). Early maturing birds have been found to have increased LYF numbers (Hocking *et al.*, 1988) and multiple LYF hierarchies (Hocking 1992) compared with late maturing birds. No difference in LYF number were observed in EARLY and LATE maturing birds in Chapter 3. However, the number of small yellow follicles (SYF), large white follicles (LWF), and medium white follicles (MWF) were increased by 51%, 39%, and 80%, respectively in LATE compared to EARLY maturing birds. The numbers of small follicles were not related to the number of LYF. Hocking (1996) stated that the probability of recruitment to the LYF hierarchy may not be closely related to the growth of pre-hierarchical follicles and that these processes may be related to different underlying physiological systems. The number of small atretic follicles at SM significantly correlated with day of first egg. Increased levels of small follicle atresia in LATE maturing birds were inversely related with LYF numbers, as expected from the observations of Hocking *et al.* (1987). The major mechanism moderating egg production is believed to be either the control of atresia within the white follicles, or the rate of recruitment to the hierarchy of yellow follicles (Hocking *et al.*, 1987). The results of Chapter 3 suggest an important role for small follicle atresia in these mechanisms.

In an experiment in which standard BW (STD), low BW (LOW), and high BW (HIGH) birds were fed either *ad libitum* (AL) or restricted fed (RF) between PS and SM, the AL feeding regimen resulted in the formation of 11.0 LYF on average compared to 7.1 in RF birds (Renema, Thesis: Chapter 5). The time from PS to SM averaged 25.3 d in AL birds compared to 38.9 in RF birds. Body size did not affect time to SM in AL birds, but in RF birds the times were 26.9, 38.4, and 51.4 d for HIGH, STD, and LOW birds, respectively. As with the EARLY and LATE birds of Chapter 3, the incidence of small follicle atresia was also greater in the later maturing birds of this experiment. The RF birds had an average of 32.3 small atretic follicles at SM compared with 10.3 in AL birds. Whereas small atretic follicles did not differ in the AL body size groups, in RF birds they ranged from 24.0 in HIGH birds to 42.2 in LOW birds. The LYF number was numerically reduced by 1.1 in RF-LOW compared to RF-HIGH birds, suggesting the previously observed inverse relationship between rate of small follicle atresia and LYF number also to be true in these birds,

In 59 wk old hens that were restricted or *ad libitum* fed (Renema, Thesis: Chapter 2), the restricted fed birds had a significantly lower apparent incidence of small follicle atresia. In birds in active egg production, higher rates of small follicle atresia appear to be associated with ovaries in a more active state of follicle production, as indicated by the overfed birds of Chapter 2 and the low VLDL-TG:PL ratio hens of Chapter 4. Hocking *et al.* (1989) did not observe any relationship between atresia in the white follicle population and LYF number at SM and speculated that a stable population of white follicles had not yet developed at or near first egg. The results of the experiments in Chapters 3 and 5 demonstrate that birds reaching SM before about 25 d after PS have lower levels of small follicle atresia than birds reaching SM later. Whereas the relationship between small follicle atresia and LYF number may be firmly established in the later maturing birds, the earlier maturing ones may be forming their LYF hierarchies according to other cues more influenced by BW.

A possible factor affecting ovarian morphology at SM is the interaction between time of exposure to elevated plasma LH and FSH concentrations with ovary maturation (Renema, Thesis: Chapter 5). Immediately following PS, plasma LH and FSH concentrations rose in all treatments. The increase in plasma estradiol-17β concentration associated with ovary development was delayed in treatments where BW or composition may not yet have met target thresholds for the onset of sexual maturation. This delay resulted in the RF birds, particularly the smaller, slower-developing ones, to be exposed to elevated plasma LH and FSH concentrations for an extended time period. The longer time period for which the ovary was exposed to high LH and FSH levels may be essential for the normal development of mechanisms controlling LYF numbers, such as the mechanism of small follicle atresia. Although birds in the AL group had significantly greater LH and FSH concentrations than RF birds due to a nutritional effect of the feeding regimen (Renema, Thesis: Chapter 5), the length of the period of elevated LH and FSH concentrations may be important as similar development was observed in populations where no birds were fed *ad libitum* (Renema, Thesis: Chapter 3).

As the potential egg production of a bird may depend partly on the state of its ovary at SM, understanding how nutritional and physiological cues interact during reproductive development is of importance to the broiler breeder industry. The experiments presented in these chapters demonstrate that certain metabolic or physical requirements must be met before egg production can commence. The size of the reproductive tract was found to be consistent within a population of birds, regardless of initial or final body size or BW. Birds that were overfed or coming into production quickly generally had more LYF, however. More unexplained post-ovulatory follicles were present in the fast maturing AL birds than in slower RF birds. Perhaps sexual maturation of the ovary occurred prior to that of the oviduct in these birds. Both plasma estradiol-17β concentration and a low VLDL-TG:PL ratio were linked to higher rates of lipid allocation to the ovary rather than to other tissues or processes. As the timing of SM has implications for ovarian morphology and possibly for how the bird will respond to feed or other exogenous challenges later in lay, these experiments indicate the need for good flock uniformity at the onset of lay. These data suggest that a flock entering lay uniformly may be easier to manage in time as they will respond to future changes in management more predictably and as a group than will birds entering lay more erratically. The lack of sensitivity of older birds to feed and estrogen challenge indicates that the link between feed intake and reproduction is less responsive than it is in younger birds.

Future research on these topics should include some strain comparisons to examine how universally theoretical BW, composition, or age thresholds exist in broiler breeders, and how they are expressed during sexual maturation. Study of how the plasma VLDL-TG:PL ratio compares in birds differing in laying efficiency and/or strain would be of interest. This would answer questions on the responsiveness of plasma lipid characteristics to change in nutritional status and on the variability of these traits between strains. Bird variability reduced the sensitivity of the measures taken in the lipid allocation and plasma lipid studies. Experiments of this nature may be improved by examining more specific markers of the traits examined, or by the more thorough examination of birds to better characterize individual responses. If the hypothalamus and/or ovary are less responsive to hormonal signaling in older birds, perhaps laying performance would be enhanced by increasing daylength or light intensity in combination with increasing feed. Monitoring FSH, LH, and VLDL size or composition in these birds would provide data on the responsiveness of the reproductive system to nutritional and environmental change.

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